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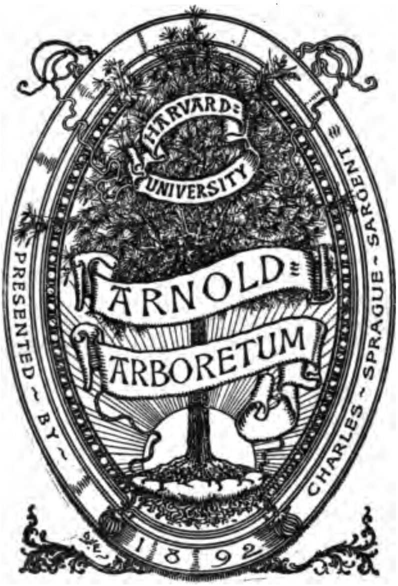
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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 100.

B. T. GALLOWAY, *Chief of Bureau.*

ISCELLANEOUS PAPERS.

I. CRANBERRY SPRAYING EXPERIMENTS IN 1905.
By C. L. SHEAR, *Pathologist.*

II. THE WRAPPING OF APPLE GRAFTS AND ITS RELATION TO THE CROWN-GALL DISEASE.
By HERMANN VON SCHRENK, *Special Agent,*
and GEORGE G. HEDGCOCK, *Assistant.*

III. GARLICKY WHEAT.
By J. W. T. DUVEL, *Assistant.*

IV. METHODS OF TESTING THE BURNING QUALITY OF CIGAR TOBACCO.
By WIGHTMAN W. GARNER, *Scientific Assistant.*

V. THE DRUG KNOWN AS PINKROOT.
By W. W. STOCKBERGER, *Expert.*

VI. ORCHARD GRASS.
By R. A. OAKLEY, *Assistant Agriculturist.*

VII. THE EFFECT OF COPPER UPON WATER BACTERIA.
By KARL F. KELLERMAN, *Physiologist,*
and T. D. BECKWITH, *Scientific Assistant.*

VIII. CONDITIONS AFFECTING LEGUME INOCULATION.
By KARL F. KELLERMAN, *Physiologist,*
and T. R. ROBINSON, *Assistant Physiologist.*

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1907.

BULLETINS OF THE BUREAU OF PLANT INDUSTRY.

The work of the Bureau of Plant Industry, which was organized July 1, 1 classified under the general subjects of Pathological Investigations, Physiol Investigations, Taxonomic Investigations, Agronomic Investigations, Hortic Investigations, and Seed and Plant Introduction Investigations. All the sci and technical publications of the Bureau are issued in a single series of bullet list of which follows.

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CONTENTS.[a]

Page.

Cranberry spraying experiments in 1905 7
  Introduction 7
  Spraying and its results 8
  Importance of early applications 9
  Effects of spraying plants when in full bloom 10
  Keeping qualities of sprayed and unsprayed fruit 10
  Cost and recommendations 11
The wrapping of apple grafts and its relation to the crown-gall disease 13
  Introduction 13
  Account of experiments 14
  Manner of wrapping 15
  Grafts left unwrapped 16
  Results of wrapping 16
    Effect upon the union 16
    Effect on crown-gall formation 18
  Recommendations 18
  Suggestion to nurserymen 19
  Summary 20
Garlicky wheat 21
  Introduction 21
  Wheat containing garlic 21
  Experiments in separating garlic from wheat 22
    Lot A 23
    Lot B 24
    Lot C 25
  The total cost of drying and cleaning garlicky wheat 26
    The net cost of removing garlic 28
  The effect of the drying on the milling qualities of the grain 28
  The effect of the drying on the vitality of the wheat 29
  Machinery used for drying and cleaning 30
  Summary 30
Methods of testing the burning quality of cigar tobacco 31
  Introduction 31
  The smoking test 33
  The effects of the filler, the binder, and the wrapper on the burn of the cigar. 35
  Testing the capacity for holding fire and the evenness of the burn 37
  Testing the burn of cigar-filler tobacco 40
The drug known as pinkroot 41
  Introduction 41
  Trade varieties of pinkroot 41
  Identity of chief substitutes 42

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[a] The eight papers constituting this bulletin were issued in separate form on February 7, February 28, April 5, June 9, October 9, October 8, October 20, and November 30, 1906, respectively.

The drug known as pinkroot—Continued.

Page.
- Minor adulterants 43
- Methods of distinguishing pinkroot from its substitutes 43

Orchard grass 45
- Introduction 45
- Methods of culture 46
  - Seeding 46
  - Mixtures with red clover 47
  - Mixtures with other grasses 4
  - Life of meadows 4
- Uses and value 4
  - Hay 4
  - Pasture 50
  - Seed 50
    - Harvesting the seed crop 51
    - Thrashing 51
    - Handling the aftergrowth 52
    - Value of the straw 52
    - Weeds in orchard grass seed fields 53
    - Other grasses in fields intended for seed 54
- Summary 55

The effect of copper upon water bacteria 57
- Introduction 57
- Resistance of various bacteria 59
- Effect of carbon dioxid on viability of Bacillus coli and Bacillus typhi 64
- Copper sulfate and filtration 69

Conditions affecting legume inoculation 73
- Introduction 73
- Use of lime 74
- Effect of soil conditions upon bacteria 76
- Effect of heavy inoculation 79
- Effect of aeration 81
- Associative action of bacteria 82
- Summary 83

ILLUSTRATIONS.

PLATES.

Page.

PLATE I. Wheat kernels and the aerial bulblets of wild garlic. Natural size.. 22

II. Fig. 1.—*A*, 1-pound sample of garlicky wheat, Lot A, as received; *B*, amount of garlic in 1-pound sample when received, 2.17 per cent; *C*, amount of garlic remaining in 1-pound sample after drying and cleaning, 0.05 per cent. Fig. 2.—*A*, 1-pound sample of garlicky wheat, Lot B, as received; *B*, amount of garlic in 1-pound sample when received, 0.56 per cent; *C*, amount of garlic in 1-pound sample after drying and cleaning, 0.06 per cent. Fig. 3.—*A*, 1-pound sample of garlicky wheat, Lot C, as received; *B*, amount of garlic in 1-pound sample when received, 2.04 per cent; *C*, amount of garlic remaining in 1-pound sample after drying and cleaning, 0.16 per cent 24

III. Variation in burn of wrappers due to different fillers 34

IV. Variation in burn of fillers due to different wrappers and binders... 36

V. Pinkroot (*Spigelia marilandica* L.) 42

VI. East Tennessee pinkroot (*Ruellia ciliosa* Pursh) 44

VII. Fig. 1.—Harvesting orchard grass for seed. Fig. 2.—Method of shocking orchard grass; shocks showing bands at top........ 50

VIII. Pots of red clover, showing the effect of lime on a soil unfavorable to the growth of clover 76

IX. Fig. 1.—Pots of garden peas, showing the effect of aeration on growth. Fig. 2.—The garden peas illustrated in figure 1, showing the effect of aeration on nodule formation 80

TEXT FIGURES.

FIG. 1. The apparatus used in the cranberry spraying experiments 8

2. Apparatus for testing the burning quality of cigars 34

3. Apparatus for testing the burning quality of wrapper tobacco 38

4. Construction of form on which leaf is wrapped for use in apparatus shown in figure 3 38

5. Cross section of the root of *Spigelia marilandica* L 44

6. Cross section of the root of *Ruellia ciliosa* Pursh 44

MISCELLANEOUS PAPERS.

I.—CRANBERRY SPRAYING EXPERIMENTS IN 1905.

By C. L. SHEAR, *Pathologist.*

INTRODUCTION.

In Farmers' Bulletin No. 221[a] a brief account was given of cranberry diseases, and also the results of spraying experiments with Bordeaux mixture. The results in 1904 were not entirely satisfactory. This was not due, however, to the inefficiency of Bordeaux mixture, but to circumstances which prevented the applications being made at proper intervals. The results obtained in 1904 showed an average of 21.7 per cent of rotten berries on the sprayed plats, as compared with an average of 76.8 per cent rotten on the unsprayed check plats. Considering the unsatisfactory manner in which the Bordeaux mixture was applied, the prediction was ventured that it would be possible with more thorough treatment to reduce the loss from rot to 10 or 15 per cent. The results obtained in 1905 have more than justified this prediction.

The experiments were conducted on what is known as the Bunker Hill bog at Whitesville, N. J., which is in charge of Mr. James D. Holman, the same plats being used as in 1904, with the addition of a small area not heretofore sprayed. This bog was selected because the fruit had in former years been almost entirely destroyed by disease. The water was drained from the bog May 10–12. It is the usual practice of cranberry growers to flood bogs for twenty-four hours during the first week in June, in order to destroy insects. In these experiments it was planned to spray part of the experimental plats before this second flooding and part immediately afterwards, in order to determine the necessity or desirability of spraying before this flooding. The water supply of the bog was, however, insufficient to flood it at the usual time, and it was not done.

The spraying apparatus used was a barrel and force pump fitted with two lengths of half-inch hose, each length provided with an extension rod and two Vermorel nozzles. The apparatus was driven about the bog in a low-bodied spring wagon, as shown in figure 1.

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[a] Farmers' Bulletin No. 221, U. S. Dept. of Agriculture, "Fungous Diseases of the Cranberry."

The Bordeaux mixture used consisted of 6 pounds of copper sulphat (bluestone) and 6 pounds of fresh stone lime to 50 gallons of water, t which was also added 4½ pounds of commercial resin-fishoil soap. Th addition of this soap has been found to be indispensable, as Bordeau mixture will not spread over and adhere satisfactorily to the glossy su face of the cranberry leaves and fruit without it. Heretofore this soa has been made as it was needed for use. Its manufacture was n an altogether pleasant operation. Now that the soap is being manu factured and placed on the market at about 3 cents per pound, it i cheaper and much more convenient to purchase it than to make it.

SPRAYING AND ITS RESULTS.

The sprayed plats were numbered 3, 5, 6, 7, and 9, the plats betwee being left as checks. Plats 3 and 7 were sprayed five times, as follow May 19, June 22–23, July 14–17, July 31–August 1, and August 15–1 On September 8, accurate counts were made of all the diseased an sound berries on small areas, showing the average condition of th berries on the sprayed plats; also of equal areas, showing the averag condition of the berries on the check plats. Plat 3 gave 3.23 per ce of rotten berries, plat 7 gave 8.8 per cent of rotten fruit, check plat showed 91 per cent of rotten fruit, and check plat 8 gave 91.53 per ce of rotten berries, giving an average of a fraction over 6 per cent of rott fruit for the sprayed plats and a little more than 91 per cent for t unsprayed plats. On these two plats it will be noted that the fir application was made on May 19, when the vines had but just co

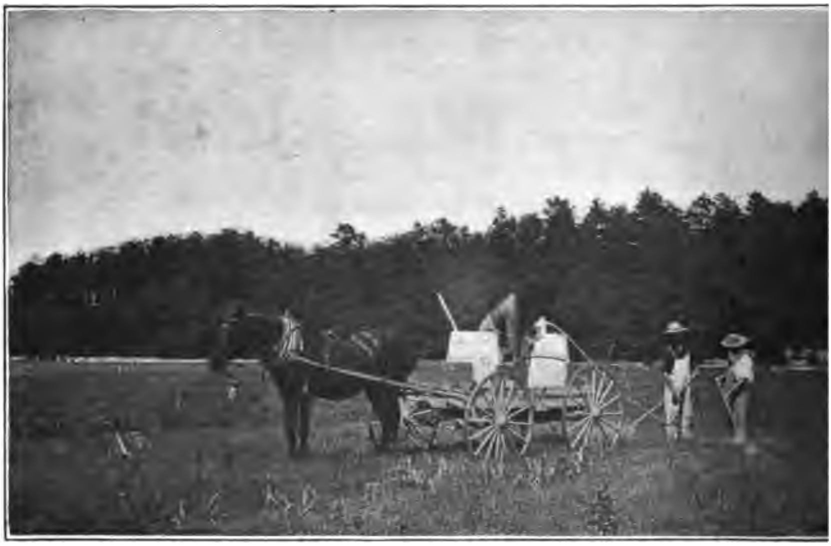

FIG. 1.—The apparatus used in the cranberry spraying experiments.

ıenced to put out [illegible] growth. This application was the one menoned as being made before the usual flooding for insects.

Plats 5 and 9 were sprayed five times, as follows: June 2, June 22–23, uly 14–17, July 31–August 1, and August 15–17. On June 2, when ıe first application was made to these plats, there was a good growth f young shoots and leaves. Counts of fruit on small areas, as in the receding case, gave the following results: Sprayed plat 5, 2.62 per ınt of rotten berries; sprayed plat 9, 2.1 per cent of rotten fruit; check lat 4, 91.8 per cent rotten; check plat 10, 93.5 per cent rotten, giving n average of 2.36 per cent of rotten berries on the sprayed plats and 2.6 per cent of rotten fruit on the unsprayed plats. There was very ttle difference in the amount of rot on this series of plats and that on ıe series mentioned in the preceding paragraph.

Plat 6 was sprayed but three times, as follows: July 14–17, July 31–ugust 1, and August 15–17. Counts made, as in the previous cases, n September 8, gave the following results: Sprayed plat, 18.3 per cent f rotten berries; check plat, 91.53 per cent of rotten fruit. It may lso be added that a greater number of the berries from this sprayed lat decayed before they were ready for shipment than was the case ith the fruit from the plats which received five applications.

The fruit from an area of 1,048 square feet, showing average condion of fruit on sprayed plat 7, was carefully hand picked and produced bushels of sound fruit, which is at the rate of about 125 bushels per re. The same area from check plat 8, showing the average condion of fruit, gave a scanty peck, or at the rate of about $10\frac{5}{12}$ bushels er acre. In other words, there was twelve times as much sound uit on the sprayed as on the unsprayed plat, or a saving of over 100 ushels per acre.

Besides our purely experimental plats, several acres upon another anberry bog known as "Long Swamp" were sprayed by Mr. Holman. ne portion was sprayed five times on the following dates: June 6–10, une 26–28, July 18–21, August 2–4, and August 18–19. One part was rayed only four times. The first plat, which was also sprayed in 904, was estimated to have from 80 to 100 per cent of the fruit sound n September 15. On the area sprayed only in 1905 it was estimated ıat from 70 to 90 per cent of the fruit was sound. On the plat which ceived the first four applications only, the fruit showed somewhat ore rot than on the other plats at picking time. The fruit on these lats had in former years been almost entirely destroyed by rot.

IMPORTANCE OF EARLY APPLICATIONS.

The difference in the appearance of the fruit on the sprayed and ısprayed plats was very marked by the middle of July. On the ısprayed plats a large proportion of the fruit was blasted, owing to the

early attack of the scald fungus (Guignardia), while on the sprayed pl
but little blasted fruit was to be seen. In many cases at least one-h
of the fruit is destroyed by blasting, the young fruits being attack
by the fungus at about the time the blossoms begin to fall. In ord
to prevent this, one of the applications of Bordeaux mixture should
made immediately after the vines have reached their maximum floweri
stage, as a delay of a week at this time may make a difference of fr
25 to 50 per cent in the amount of fruit destroyed by blasting.

A very striking illustration of this fact was observed upon anoth
bog where one plat had been sprayed on July 1 and another adjoini
was not sprayed until July 8. On the plat sprayed on July 1, wh
the vines had just reached their maximum flowering condition, b
very little blasted fruit could be found, whereas on the plat which h
been sprayed on July 8 about one-half of the fruit had been blaste
This and other observations indicate the exceedingly great importan
of prompt and thorough early applications of the fungicide. In ca
the bog to be sprayed is flooded for insects early in June, the fi
application of Bordeaux mixture should be made within a day or t
after the water is removed, the second application just as the plan
begin flowering, and the third just after the majority of the blosso
have appeared, which in ordinary seasons will be about the first of Ju

EFFECT OF SPRAYING PLANTS WHEN IN FULL BLOOM.

In order to determine whether any injury would result from sprayi
the plants while in bloom, part of one plat was sprayed when in f
bloom, and the amount of fruit which set upon this plat was careful
compared with that on adjoining plats which had not been spraye
No difference could be noted in the amount of fruit on the sprayed a
on the unsprayed plats. In addition, certain bunches of vines we
dipped in Bordeaux mixture when in full bloom, but without a
apparent injury to the fruit. From our observations and experimen
it does not appear that there is much danger of loss from spraying vi
while in bloom. What little loss might possibly arise from this ca
would be very slight compared with the amount of loss from blast
fruit in case the spraying was delayed too long.

KEEPING QUALITIES OF SPRAYED AND UNSPRAYED FRUIT

A comparison of the sprayed and unsprayed fruit at the time
picking does not give an exact idea of the amount of profit deri
from the treatment, as there was a much greater loss of unsprayed th
of sprayed fruit between the time of picking and the time the berri
were marketed.

In order to compare the keeping qualities of the sprayed a
unsprayed fruit, as well as unsprayed fruit which had been treat

ith a solution of copper sulphate (1 part to 1,000 of water), 3,600
rries, perfectly sound so far as could be seen from external appear-
ce, were selected from fruit picked on September 18, 1905. Of these,
200 were from the sprayed plats and the remainder from the unsprayed
eck plats. These berries were kept in glass dishes in the laboratory
d counted each week, in order to determine the amount of disease
hich developed. On October 18, about the time the fruit from the
g was marketed, 9.8 per cent of the sprayed fruit showed diseased
rries, while 38.1 per cent of the unsprayed fruit and 37.4 per cent of
e unsprayed fruit which had been treated with the copper-sulphate
lution were diseased. In other words, four times as much of the
sprayed fruit decayed between the time of picking and marketing as
the sprayed fruit.

Parts of the check plats which were worth picking were picked by
nd and the fruit kept separate. This fruit was sorted for shipment
October 15, 1905, when from 85 to 90 per cent of it was rotten. Mr.
olman states that 40 per cent of all the unsprayed berries from Bun-
er Hill bog decayed between the time of picking and marketing. No
eatment of the berries with fungicides after picking is likely to give
tisfactory results, as we have found that the decay which occurs in
orage does not arise from germs which are on the surface of the fruit
the time it is picked, but apparently from a dormant form of the
ngus already within the berries, where it is awaiting favorable con-
itions for development.

As the time for picking approached, so much of the Bordeaux mix-
re adhered to the sprayed fruit that it was feared enough might be
resent when the berries were marketed to interfere with their sale.
his, however, did not prove to be the case, as the greater portion of
e mixture was removed from the fruit during the processes of pick-
g, sorting, and preparing the fruit for market.

As the result of three years' spraying experiments, it is safe to say
at by the proper use of Bordeaux mixture the loss from fungous dis-
ses can be reduced to 10 per cent or less. The loss may be slightly
ore the first year a badly diseased bog is treated, as the benefit, as
hown in these experiments, is greater the second year than the first,
nd is evident not only in the prevention of scald and rot of the fruit,
ut in the general improvement, thriftiness, and productiveness of the
ines. Bordeaux mixture has also been applied with very beneficial
esults to young vines not yet in bearing, the leaves of which were
adly affected by the scald fungus.

COST AND RECOMMENDATIONS.

The cost of the spraying as it was done in these experiments averaged
rom $15 to $20 an acre, the mixture being applied at the rate of four

barrels, or 200 gallons, an acre at each application, making for the
applications a total of 1,000 gallons to the acre.

In undertaking the application of Bordeaux mixture it is necess
that all material and apparatus be in perfect readiness before the ti
to begin the work, and nothing should be allowed to interfere with
application of the fungicide at proper intervals; otherwise, the resu
will be unsatisfactory, and the remedy is likely to be unjustly c
demned.

The necessity for care and thoroughness in the preparation and ap
cation of the mixture must also be strongly emphasized. The mixtu
should be made as described in Farmers' Bulletin No. 221, using o
good, fresh stone lime and adding resin-fishoil soap. A good noz
(the Vermorel type is best) should be used and the vines very th
oughly covered at each spraying. Four barrels of the mixture to
acre are ordinarily sufficient, but where the vines are very dense
heavy five barrels may be necessary. Where there is an excess
growth of vines, it will be found advantageous to rake them wit
knife rake and thin them out, as is frequently done in order to prep
a bog for picking with scoops.

. I.—203.

–THE WRAPPING OF APPLE GRAFTS AND ITS RELATION TO THE CROWN-GALL DISEASE.

IERMANN VON SCHRENK, *Special Agent in Charge of the Mississippi Valley Laboratory,*
and GEORGE G. HEDGCOCK, *Assistant in Pathology.*

INTRODUCTION.

he crown-gall disease of apple trees, as indicated in a recent pub-
tion of the Bureau of Plant Industry,[a] appears in three distinct
es—the hard crown-gall, the soft crown-gall, and the hairy-root
ns. While the hairy-root disease of apples has usually been con-
red a type of the crown-gall disease, it in reality is an entirely
erent trouble, with manifestations which are not in the least like
e met with in the true crown-gall disease. The soft type of crown-
on the apple has not yet been clearly differentiated from the hard
, and in the following discussion the two are considered as one.

he knots which characterize the crown-gall disease of the apple
lly appear at some point, either on the root piece or on the scion,
re the two are united; that is, the gall may form at the end of the
ue of either the scion or root piece, or at any point where either
e has been wounded. This fact is one commonly recognized by
urserymen and scientific workers who have studied this disease.
ut 90 per cent of these knots will appear on the end of the scion
e. The exact cause of the formation of the gall is as yet somewhat
rtain. It would seem, however, that, whatever the cause, the
t most exposed to the disturbing factor, whether it be due to a
us, to bacteria, or to soil or atmospheric conditions, is the junction
e scion and root piece.

hen the newly made grafts are laid away in the grafting cellar
r in sawdust, excelsior, moss, or other bedding material, callous
e begins to form on the cut surfaces of both scion and root piece.
callous tissue from the two pieces will in time fill the intervening
es between the surfaces of the scion and the root piece, and ulti-
ly the root callus and the scion callus will join. Where the root
and the scion are of exactly the same size and where they are
d so as to fit exactly, a very perfect union will take place. Where

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Crown-Gall and Hairy-Root Diseases of the Apple Tree," 1905.

there is a difference in size between the root piece and the scion, where they are more or less imperfectly fitted, as is almost always t case in commercial grafts, the callus formed by the scion and the r piece will not always meet along equal planes. In these cases th will be a tendency for the callus forming on any surface of either pi which is not in direct contact with an opposite surface (no matter h small this surface may be) to grow out from this surface, either lat ally or down or up, into the air, with nothing to prevent its growi into small lumps, which may reach considerable size. Very frequen the pressure exerted by the growing callus will push the tongue of graft outward. A thick cushion of callus will then form between scion and root piece.

The object of all grafting should be to bring about as rapid a uni between the two grafted parts as possible, with the smallest possi period during which an open wound is allowed to remain into wh disturbing factors may enter. With this in mind, a number of exp ments were made during the past year, the specific objects of wh were to confine the formation of callus as far as possible to the spa between the root and scion pieces, thereby bringing about a closer more effective union and reducing the chances for the entrance possible disturbing factors.

It is intimated that the formation of the hard and soft types of ap crown-gall may be due either to a proliferation of callous tissue in ca where an uneven union is taking place and where the callus has h chance to grow out laterally in an unrestricted manner, or to fu bacteria, or unfavorable soil or atmospheric conditions. Whatever cause, the making of a perfect union in the shortest possible time serve to prevent the trouble almost wholly if the first explanation gi be the cause, and at least largely remove the danger of a possi infection if the second explanation is found to hold good.

ACCOUNT OF EXPERIMENTS.

In order to confine the formation of the callus, a number of gr were wrapped with various materials during the winter of 1904–5. wrapping idea is no new one, because cloth and paper were used nurserymen many years ago in connection with various types of r and top grafting. At the present time cloth and paper are used v little, because the system of thread wrapping will give a larger num of grafts in a given period of time. The materials used in our periments were cloth, thin sheet rubber (dental rubber), and wa paper. In addition to this, grafts were made with plain thr and waxed thread, and some grafts were wrapped with plain thr and afterwards the whole union was covered with grafting w One series of grafts was made without any wrapping of cloth ead whatever, the pieces being simply fitted together with e. All grafts made were of the type known as tongue or whip ıfts. The work was done by an expert, so that all of grafts may be considered as especially well made. No. 1 Kan- roots and a selected lot of scions of the following varieties were ıd: Winesap, York Imperial, Wealthy, Missouri, and Northern Spy. e grafts were made in February, 1905. They were stored in chopped ıelsior for about six weeks, were then planted in eight different locali- ı in Missouri, Illinois, Iowa, Nebraska, Kansas, and Arkansas, and en the usual cultivation during the summer. All showed a fair stand November, 1905. Some of the grafts were dug in November, and ;ember, 1905, one-third of the total number planted in five test plats four States being dug. The results obtained are given later.

MANNER OF WRAPPING.

:he manner in which the wrapping was made and the material used. y be briefly described as follows:

?loth.—The cloth used was a cheap black calico of the poorest ıde obtainable in the market. This was torn into strips 1 inch wide 4 or 5 inches long. After the graft was made, one end of the cloth ı dipped for one-half inch into hot melted grafting wax. Starting h the other end, the cloth was then wrapped tightly around the scion the free waxed end pressed down. This completed the operation.

?ubber.—The rubber used was of a quality similar to dental rubber, ch is also frequently used for insulating wires. It was bought in s 1 inch wide, and was cut off in such lengths as were necessary to ıpletely envelop the union. The rubber was usually wrapped so as ;o around the union twice, and the free end was fastened with rubber ıent applied with a brush.

Vaxed paper.—Sheets of ordinary unglazed printers' paper were :ed on one side by coating them with hot grafting wax applied with ıint brush. They were then cut into inch strips, 10 to 20 sheets ıg cut at one time, and these again into strips about 4 or 5 inches ;. One paper strip was then wrapped around the union, the waxed toward the graft, the free end being stuck down by pressing it on.

'lain thread.—The thread used for the ordinary grafts was a hine cotton, No. 9.

Vaxed thread.—The thread used was a machine cotton, No. 28. ; thread was soaked in hot grafting wax until thoroughly penetrated was then allowed to drain while hot.

'lain thread with union waxed.—These were ordinary grafts made ı plain thread, wrapped as previously described, and then coated ı melted grafting wax nearly at the point of hardening.

GRAFTS LEFT UNWRAPPED.

The grafts left without wrapping of any kind were made with sp care, so that when the roots and scions were joined they rem firmly united.

RESULTS OF WRAPPING.

After digging the grafts, a careful examination of the various s was made to determine the effect of the wrapping on the union an the presence or absence of crown-gall formation.

The following table shows the number of apple trees dug, the c acter of the wrapping, and the number and percentage of smooth t and rough trees, the latter class including all trees which were f to have crown-gall, irregular callus, or hairy-root formations:

TABLE 1.—*Comparison of smooth trees and rough trees resulting when different me of grafting were used.*

Kind of wrapping.	Total number.	Smooth trees.		Rough tre	
		Number.	Per cent.	Number.	Per
Rubber	675	584	86.5	91	
Cloth	709	604	85.1	105	
Waxed paper	671	474	70.6	197	
Plain thread	645	442	68.5	203	
Waxed thread	675	430	63.7	245	
Plain thread with union waxed	402	178	44.2	224	
Unwrapped	569	312	54.8	257	

Two series of facts may be deduced from this table—

(1) The effect of the various kinds of wrapping on the smoothn of the union.

(2) The effect of wrapping on the presence or absence of crown-g formations.

EFFECT UPON THE UNION.

Some brief notes on the effect of the wrapping on the union follo By a smooth graft is meant one which shows no uneven or irregu masses of callus. In the smooth grafts all parts are firmly united, that no ridge or roughness is felt when the union is rubbed with fingers. The grafts were graded with the greatest care.

Cloth.—When the grafts were planted in the spring the cloth usually intact. When dug in the autumn it had almost entirely rot away on all grafts. The union which resulted under the cloth generally smooth and very even. In many cases it was difficult detect the original position of the scion and root parts. The cloth evidently remained in position long enough to thoroughly confine callus to the spaces between the wounded surfaces.

Rubber.—When the grafts were planted the rubber wrapping wholly intact. When dug the rubber was still found around the un the majority of cases. It had, however, exerted no injurious effect, ause, as the young trees had grown in diameter, the rubber had panded. The roots had frequently punctured the rubber freely. other words, the rubber had in no way retarded the growth. The ions formed under the rubber were smooth and even; in fact, they re the most perfect of all those formed in the experiment. The bber covering was practically waterproof. So perfect were the ions that in many cases it was possible to detect the line of demar-ion between the root and scion only by the difference in color.

Waxed paper.—When planted in the spring the waxed paper had not ted, but it had a tendency to become unrolled, due probably to too ch oil in the wax. When dug in the autumn the paper had mostly appeared. The grafts showed many smooth trees, but the percentage s not nearly so large as in the case of those wrapped with rubber or th. Attention should be called to the fact that in all grafts where x was used the wax itself seemed to exert an unfavorable effect on union, and the writers are inclined to discourage its use wherever omes into direct contact with wounded surfaces.

Plain thread.—When planted in the spring the plain thread had in ny instances entirely rotted away. The grafts when dug showed ny callus proliferations. The percentage of smooth grafts was low en compared with rubber or cloth wrappings. The results were out as good as those secured by the use of waxed paper wrapping, d a little better than those obtained with waxed thread.

Waxed thread.—When the grafts were planted the waxed thread was act. When dug in the autumn the grafts showed from 6 to 8 per nt with knots caused by the pressure exerted by the thread, which d not rotted away because the wax protected it more or less. The rcentage of smooth trees aside from this was almost the same as for plain thread. No perceptible advantage appeared to be gained by use of waxed thread as compared with plain thread.

Plain thread with union waxed.—At the time of planting it was noted t the wax had in many instances penetrated between the surface of two pieces. The callus had formed in irregular masses, there being thing to confine it. It furthermore appeared as if the wax had exerted unfavorable effect already referred to in connection with the use of xed paper. When dug the trees in the majority of instances showed ead knots, due no doubt to the fact that the wax had retarded the ting away of the thread. The unions were usually rough. The favorable effect ascribed to the wax was evident not only in the mediate wound areas, but also all along the bark on both scion and ot pieces, wherever the wax came in contact with the tissue.

Unwrapped grafts.—The grafts with no wrapping showed the excess- development of callus more than any others. However, many of the resulting trees when dug in the autumn showed fairly good unio The grafts as a whole fell below the wrapped ones in the number smooth trees. There were more deficient trees in this lot, with t possible exception of those waxed over, owing to defective unions a failures to form a union.

EFFECT ON CROWN-GALL FORMATION.

As indicated in the introduction, one of the objects of the experim was to determine whether by wrapping grafts it would be possible reduce the number of trees affected with crown-gall. In the followi table the total number of trees dug is given; also the kind of wrappin and the number and percentage of smooth trees, crown-gall trees, a hairy-root trees:

TABLE II.—*Comparison of smooth trees and trees affected with crown-gall and hairy-r resulting when different methods of grafting were used.*

Kind of wrapping.	Total number.	Smooth trees.		Crown-gall trees.		Hairy-root tre	
		Number.	Per cent.	Number.	Per cent.	Number.	Per c
Rubber	675	584	86.5	65	9.6	26	
Cloth	709	604	85.1	70	9.8	35	
Waxed paper	671	474	70.6	168	25.0	29	
Plain thread	645	442	68.5	130	20.2	73	
Waxed thread	675	430	63.7	182	27.0	63	
Plain thread with union waxed	402	178	44.2	193	48.1	31	
Unwrapped	569	312	54.8	200	35.2	57	

A study of this table will show that the wrapping reduced the numb of crown-gall trees very materially, but the results given should considered as preliminary and the figures as relative rather than ab lute. Only a small number of the trees under test have so far b dug. The remainder will be dug after one or two years' growth.

The most effective wrapping, so far as the true crown-gall is c cerned, was that made of rubber (86.5 per cent of smooth trees), f lowed closely by cloth (85.1 per cent of smooth trees). The cloth wr ping, however, shows the highest percentage of smooth trees per grafts planted when not only the crown-gall but also the hairy-r are considered. The difference is very slight, however. The o wrappings show less favorable results, least of all in the case of grafts wrapped with plain thread and covered with grafting wax (4 per cent of smooth trees), followed closely by those with no wrapp whatever (54.8 per cent of smooth trees).

RECOMMENDATIONS.

From the results so far obtained, the use of either cloth or rubb material for wrapping apple grafts is recommended. Owing to expense involved in the use of rubber, cloth will be found the desirable, and in most cases will probably give results fully as satis ry as rubber. The writers strongly advise against the wrapping of rafts with thread and subsequently waxing the grafts.

SUGGESTION TO NURSERYMEN.

The results obtained from the experimental plats having shown that e wrapping has materially reduced the number of crown-gall and other pes of rough trees, it will be very desirable to test these results on a rger scale. The grafts in these experiments were all made with the eatest care, and it would seem probable that the number of smooth ees obtained was therefore larger than might be the case where comercial grafts are made. The latter are usually not fitted with as much re, either with respect to size of scion and root pieces or with regard an even and close union in the freshly made graft. It is therefore rged that nurserymen generally test the wrapping of their grafts is winter, either with rubber or cloth, after the manner previously scribed.

Care should be taken in setting out grafts with different kinds of rapping to treat them in the same manner, that is—

(1) Use the same variety of scion.
(2) Use the same stock of roots.
(3) Make the grafts at the same time.
(4) Plant the grafts on the same date.
(5) Plant in the same field or at least on similar soil.
(6) Cultivate all grafts alike.

Where such tests are made, it is requested that the Bureau of Plant dustry be informed, so that all the experiments may be compared.

It may prove of interest to estimate the total number of smooth and ugh trees to the acre which would result from the use of various rappings, estimating 18,000 grafts to the acre and using as a basis the rcentages of such trees obtained in the tests described in these pages. y smooth trees are meant trees free from crown-gall and other disses; by rough trees, trees affected with true crown-gall, hairy-root, manifestations of other diseases at or near the point of union. This timate has been attempted in the following table, which should be garded only as suggestive, however. The results are averaged from e identical plats in four different States, containing about 9,000 grafts.

BLE III.—*Estimate of the number of smooth trees and rough trees which can be raised on an acre of land by using various methods of grafting.*

Wrapping.	Smooth trees.	Rough trees.	Total trees.
bber	12,600	1,980	14,580
th	12,960	2,340	15,300
xed paper	10,260	4,320	14,580
in thread	9,540	4,320	13,860
xed thread	9,360	5,220	14,580
in thread with union waxed	3,780	4,860	8,640
Unwrapped	6,660	5,580	12,240

SUMMARY.

(1) The crown-gall disease of apple trees usually appears at or ne the union of the scion and root piece.

(2) The crown-gall disease may be due to an excessive growth o callus, or to an infection of fungi, bacteria, or other agencies at or ne the union.

(3) Protection of the graft at the union will serve to induce a bett union and may also aid in keeping out disturbing factors.

(4) In making grafts care should be taken to use root and scio pieces of as nearly the same size as possible.

(5) Grafts wrapped with cloth and with rubber yielded 85.1 per cen and 86.5 per cent of smooth trees, respectively.

(6) Ordinary thread grafts yielded 68.5 per cent of smooth tree plain thread grafts with the union waxed, 44.2 per cent; grafts wit no wrapping, 54.8 per cent.

(7) It is recommended that apple grafts be wrapped with cloth o rubber.

P. I.—204.

III.—GARLICKY WHEAT.

By J. W. T. Duvel, *Assistant in the Seed Laboratory.*

INTRODUCTION.

Wild garlic (*Allium vineale* L.) was introduced into the United tates from Europe considerably more than a century ago. Since its troduction it has made a slow but steady advance, and is now und growing more or less abundantly throughout the greater part West Virginia, Virginia, Maryland, Delaware, Tennessee, North rolina, the northern part of South Carolina, the southern part of ennsylvania, New Jersey, and Connecticut, and locally in almost ery State east of the Mississippi River. In all places where it has ecome well established it is a veritable pest to farmers, millers, grain ealers, and dairymen.

Wild garlic is one of the worst weeds to eradicate after it has once ined a foothold, being propagated by underground bulbs, aerial lblets, and in some sections by seeds.

WHEAT CONTAINING GARLIC.

The presence of wild garlic in the grain fields of the central eastern ates and in other sections where it is locally abundant has caused a ry great loss to agriculture. Farmers have been obliged to sell their rlicky wheat at greatly reduced prices, principally because foreign rkets will not buy it except at a low price, and millers as a rule use to handle it, for they have been able to grind garlicky grain only a much increased cost. The garlic bulblets gum the rollers, neces- ating the stopping of the mills and the washing of the rollers before e grinding can be resumed. The frequency with which the washing st be done depends on the quantity of garlic present. In extreme ses the washing must be repeated every two or three hours, the eration requiring from ten to fifteen minutes for each set of rollers. Furthermore, flour made from wheat mixed with garlic bulblets is of erior quality, as bread made from such flour has the garlic odor so agreeable to most people. This is especially noticeable if the ead is eaten warm. Moreover, on boards of trade, wheat containing lic bulblets in considerable quantity is graded as "Rejected," and hen sold only on sample. Wheat of this character is generally sold a price ranging from 20 to 40 per cent lower than No. 2 Red. However, if the garlic bulblets are present only in comparatively sma quantity (usually less than one-fourth of 1 per cent) it may pass No. 2 Red, depending largely on the other foreign substances prese and the amount of water in the grain.

At present there are no available data showing definitely the exte of the loss due to the presence of garlic in grain; but in wheat alo this loss is known to be very great. In many sections the growing wheat has been almost wholly abandoned as a result of the reduc price at which garlicky wheat must be sold. An annual loss $1,500,000 is undoubtedly a very conservative figure. It has bee estimated by members of the Chamber of Commerce of Baltimore th 60 per cent of the wheat grown in that section of the United Stat contains more or less garlic. The three States in which garlic do the greatest injury to the wheat crop are Maryland, Virginia, a Tennessee. The average yield of wheat from these three Stat during the five years from 1900 to 1904, inclusive, was just short 29,000,000 bushels. Allowing that 50 per cent of this wheat contai garlic, we have 14,500,000 bushels of garlicky wheat in these thr States alone. But granting that only one-half of this amount co tains garlic in sufficient quantity to throw it out of grade, we sti have 7,250,000 bushels of wheat which must be sold at a great reduced price. A reduction of only 15 cents per bushel means mo than a million dollars annually to the farmers of Maryland, Virgini and Tennessee. The members of a prominent firm of grain export in Baltimore state that the depreciation for the Maryland crop alon which amounts to about 12,000,000 bushels annually, will be fully to 10 cents per bushel, or an equivalent of $600,000 to $1,200,000. large quantity of garlicky wheat, however, does not get into the e vators, being fit only for feeding purposes. Mr. R. L. Wells[a] stat that, in Tennessee, wheat containing garlic bulblets has been sold low as 15 cents per bushel to feed stock.

EXPERIMENTS IN SEPARATING GARLIC FROM WHEAT.

The presence of the aerial bulblets of wild garlic in wheat has alwa been objectionable, principally because of the extreme difficulty separating them from the wheat. While some of the lighter, imm ture bulblets can be blown out by a good fanning mill, the great number are of practically the same size and weight as the wheat ke nels. Plate I shows wheat kernels and the aerial bulblets of wild g lic of natural size. This similarity in size and shape makes it imp sible to separate them during the autumn or early winter by the use the ordinary cleaning machinery usually found in the majority of flo mills and elevators, i. e., by screening and fanning. If the bulbl

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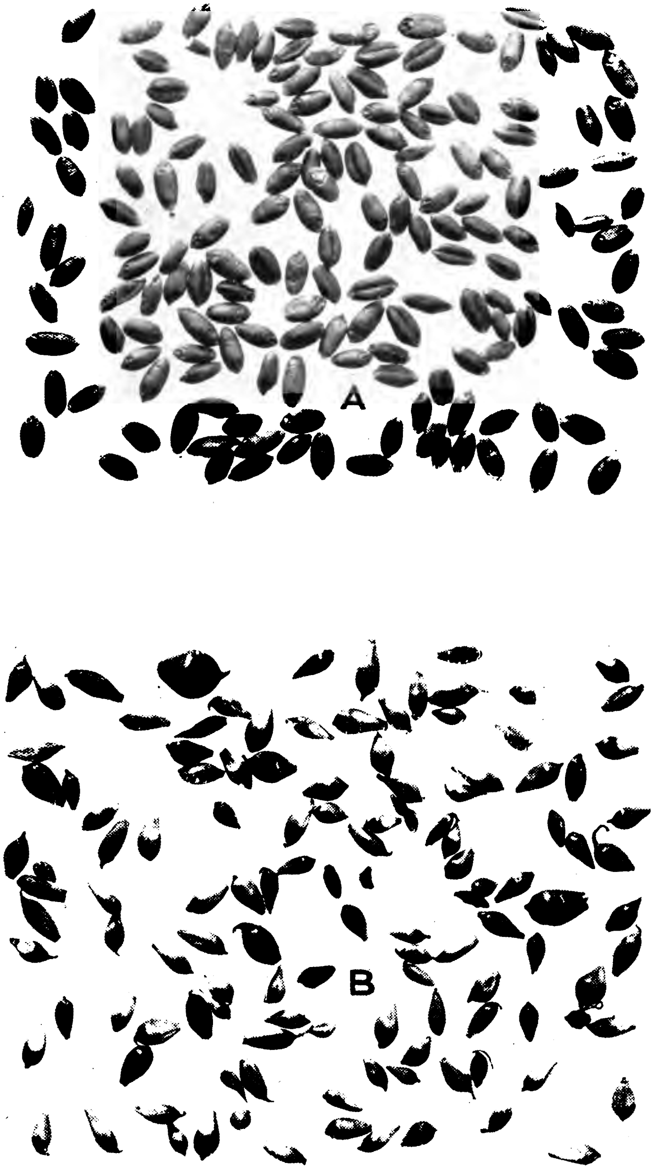
[a] The Wild Onion, Bulletin, Tennessee Agricultural Experiment Station, July, 1895.



WHEAT KERNELS (A) AND THE AERIAL BULBLETS OF WILD GARLIC (B).

(Natural size.)

e allowed to freeze, they afterwards become dry and are then quite adily blown out, but this is not always practicable.

In view of this fact experiments were undertaken in June, 1905, in der to ascertain whether the mixture of garlic and wheat could not dried artificially, thereby reducing the weight of the bulblets to ch an extent that they could be satisfactorily removed as soon as e grain is ready for market. The detailed results of these experi- ents[a] are given in the following pages.

LOT A.

Lot A consisted of approximately 44 bushels of "rejected" wheat rnished by the Baltimore Chamber of Commerce. When received contained 16.55 per cent of water and 2.17 per cent of garlic. The ount of foreign seed and chaff present was not determined. The lue of this wheat was placed at 65 or 70 cents a bushel.

Experiment No. 1.—A portion of this wheat was dried in the small ain drier of the Seed Laboratory at a maximum temperature of 136° for two hours. During this time the moisture content of the grain s reduced from 16.55 per cent to 9.5 per cent, or from 2½ to 4½ per nt less than good American wheat normally contains. But this gree of drying proved insufficient, as 0.28 per cent of garlic still nained in the sample after a preliminary cleaning. This same lot of eat was therefore dried for an additional half-hour and the mois- re content was reduced to 8.94 per cent.

Experiment No. 2.—Another portion of seed from Lot A was dried ew days later for nearly four hours, the maximum temperature ding 140° F. At the termination of the drying a moisture deter- nation of a sample of this wheat showed only 5.87 per cent of water.

The wheat from experiments Nos. 1 and 2 was then mixed and aned, and the average percentage of water in the mixed sample was nd to be 7.41 per cent. After cleaning, an analysis of this wheat wed that the amount of garlic had been reduced from 2.17 to 0.05 cent, 97.6 per cent of the garlic having been removed. Plate II, ure 1, shows a 1-pound sample of this wheat as received, the antity of garlic in 1 pound when received, and the quantity of lic remaining in 1 pound after drying and cleaning.

oncerning this lot of wheat the secretary of the Baltimore Cham- of Commerce wrote as follows:

> he wheat which you cleaned and returned was the source of a great deal of interesting ment upon the floor of the chamber, and the general idea is that a very vast change was mplished by running it through the drier. The sample sent originally was of such low

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[a] cknowledgments are due to the members of the Baltimore Chamber of Commerce and r. Walter Roberts, of Alexandria, Va., and Mr. E. H. Darby, of Seneca, Md., who kindly plied the garlicky wheat for these experiments.

and inferior grade as to prohibit it from going into the elevators, and the drying and cl to which it was subjected made it No. 2 Red, the contract grade, a difference in v fully 17 cents per bushel.

An increase of 17 cents per bushel was equivalent to 24.6 per of the value of this wheat before drying and cleaning.

LOT B.

A second sample of approximately 38 bushels of "rejected" w furnished by Mr. Walter Roberts, Alexandria, Va., contained 0.5 cent of garlic and 15.08 per cent of water and weighed only 57.5 po per bushel.

The lot was divided into three parts for treatment, as follows:

Experiment No. 3.—In this test the drying was continued for t hours, the temperature of the air varying from 153° to 158° F. an temperature of the wheat from 117° to 155° F. The moisture wa duced from 15.08 to 7.92 per cent. The weight per bushel was creased from 57.5 to 59.25 pounds on drying and to 60.6 pounds cleaning. The quantity of garlic was reduced to 0.05 per cent, same as the combined results of experiments Nos. 1 and 2.

Experiment No. 4.—The period of drying in this experim extended over three and one-half hours, the temperature of the being the same as in experiment No. 3, 153° to 158° F., the temp ture of the wheat gradually increasing from 95° F. at the end of first half-hour to 145° F. During the three and one-half hours' dry the water content of the wheat was reduced to 6.88 per cent and weight per bushel increased to 59.5 pounds. After cleaning weight per bushel was increased to 60.7 pounds and the quantity garlic reduced from 0.56 per cent to 0.06 per cent. Plate II, figu shows a 1-pound sample of this wheat as received, the amount of in 1 pound when received, and the amount of garlic remainin pound after drying for three and one-half hours and cleaning.

Experiment No. 5.—The last portion of Lot B was dried for t three-fourths hours, the temperature of the wheat reaching 122 three-quarters of an hour, and 138° F. after one hour, which te ture was maintained for one-half hour, gradually decreasing the last one and one-quarter hours to 117° F., when the exp was concluded. The moisture content of the wheat was reduc 15.08 to 8.48 per cent and the weight per bushel raised from 58.6 pounds. After cleaning, the weight per bushel was 60 and the garlic present 0.07 per cent.

After drying and cleaning, the wheat from Lot B graded No. having at that time a value of 85 cents per bushel. As in its o condition the wheat was purchased for 55 cents per bushel, the and cleaning increased its value 54.5 per cent.

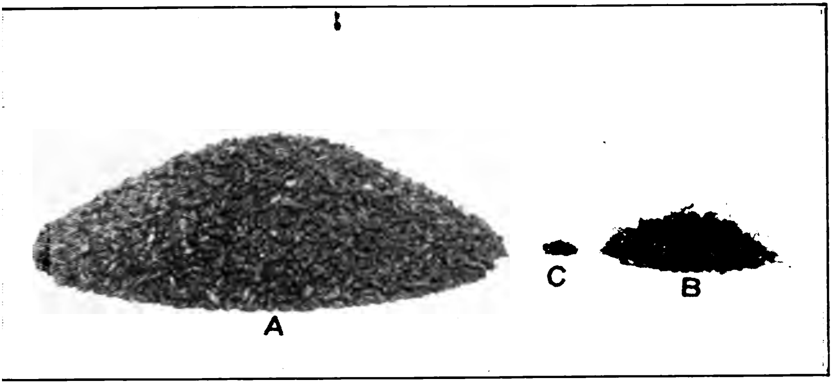


FIG. 1.—WHEAT AND GARLIC FROM LOT A.

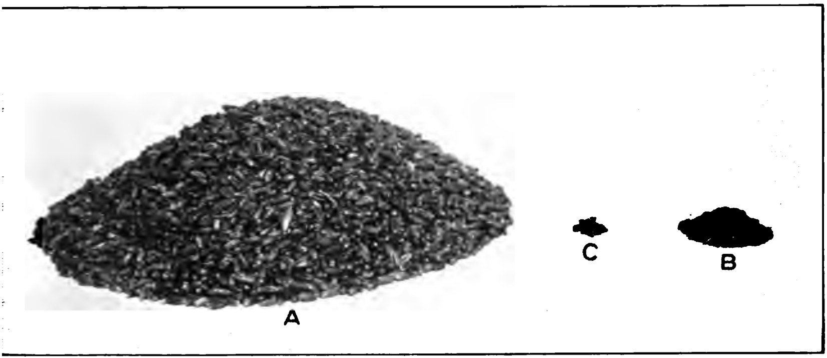


FIG. 2.—WHEAT AND GARLIC FROM LOT B.

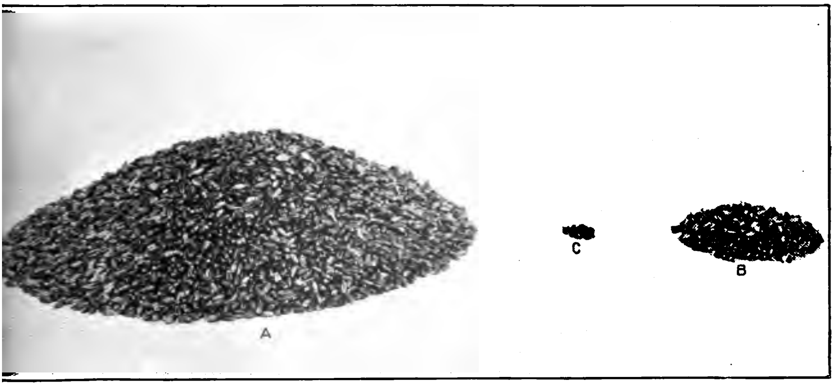


FIG. 3.—WHEAT AND GARLIC FROM LOT C.

ONE-POUND SAMPLES OF GARLICKY WHEAT AS RECEIVED (A, A, A); THE QUANTITY OF GARLIC IN ONE-POUND SAMPLES WHEN RECEIVED (B, B, B); THE AMOUNT OF GARLIC REMAINING IN ONE-POUND SAMPLES AFTER DRYING AND CLEANING (C, C, C).

LOT C.

A consignment of approximately 30 bushels of "rejected" wheat, ntaining 2.04 per cent of garlic, 16.2 per cent of water, and weigh- g only 56.5 pounds a bushel, was lent to the Department of Agricul- re by Mr. E. H. Darby, of Seneca, Md.

This lot of wheat was divided into two parts and treated as experi- ents Nos. 6 and 7.

Experiment No. 6.—This wheat was subjected to an air temperature 113° F. for one hour and of 154° F. for two hours, the maximum mperature of the grain for the last half hour being 149° F. The mois- re content was reduced from 16.2 per cent to 8.2 per cent. The eight per bushel was raised to 57.8 pounds after drying and 60.6 ounds after cleaning, and the amount of garlic was reduced to 0.17 er cent.

Experiment No. 7.—This experiment continued for three hours, as experiment No. 6, but the temperature of the air current decreased radually from 146° to 122° F., the maximum temperature of the rain being 131° F. Samples taken at the termination of the experi- ent showed a moisture content of 8.83 per cent. The weight per ushel was increased to 57.5 pounds after drying and 60.2 pounds ter cleaning. Plate II, figure 3, shows a 1-pound sample of this heat as received, the amount of garlic in 1 pound when received, and e amount of garlic remaining in 1 pound after drying for three ours and cleaning.

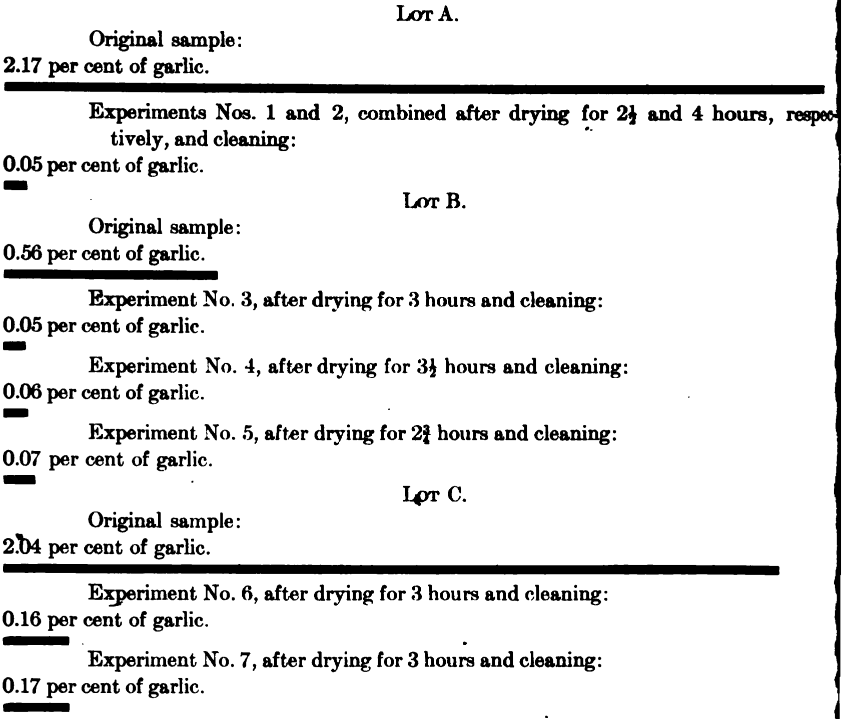
A sample of this cleaned wheat was examined by the chief inspector the Baltimore Chamber of Commerce and graded as No. 2 Red, giv- g it a value of 84.5 cents per bushel. The highest price offered for e original lot of wheat was 60 cents per bushel. The removing of e garlic and the cleaning consequently enhanced the value 40.8 per nt.

In experiments Nos. 6 and 7 the drying was not continued quite ng enough for the best results, although the quality of the wheat as raised to "contract" grade. At temperatures from 150° to 158° . the drying should continue for two and one-half to three hours, or ntil the moisture content of the wheat is reduced to about 8 per cent.

In none of the experiments was it possible to remove all of the garlic, ut in every case the quantity was reduced considerably more than as necessary to make the wheat grade as No. 2 Red. Moreover, the uantity of garlic present after the cleaning was not considered suffi- ent to interfere with the milling of the wheat or to injure the quality the flour.

The following diagrammatic figures show the relative quantity o garlic in the wheat before and after treatment:

Percentages, by weight, of garlic in wheat, Lots A, B, and C, before and after drying and cleani



LOT A.

Original sample:
2.17 per cent of garlic.

Experiments Nos. 1 and 2, combined after drying for 2½ and 4 hours, respectively, and cleaning:
0.05 per cent of garlic.

LOT B.

Original sample:
0.56 per cent of garlic.

Experiment No. 3, after drying for 3 hours and cleaning:
0.05 per cent of garlic.

Experiment No. 4, after drying for 3½ hours and cleaning:
0.06 per cent of garlic.

Experiment No. 5, after drying for 2¾ hours and cleaning:
0.07 per cent of garlic.

LOT C.

Original sample:
2.04 per cent of garlic.

Experiment No. 6, after drying for 3 hours and cleaning:
0.16 per cent of garlic.

Experiment No. 7, after drying for 3 hours and cleaning:
0.17 per cent of garlic.

Garlic bulblets as found in wheat contain from 35 to 70 per cent o water, while the water content of fresh garlicky wheat usually vari from 15 to 20 per cent. During the drying the amount of water in th wheat is decreased, but at the same time the kernels become more compact and the specific gravity is increased, as is shown by the weigh per bushel before and after drying. On the other hand, the specifi gravity of the garlic bulblets is lowered by the drying. The oute membranous coverings of the bulblets remain distended and th shrinkage takes place in the inner portion, thus leaving a small ai space between the bulb proper and the outer protecting layers. Thi increased air space, together with the decreased weight due to the los of water, makes it possible to separate most of the bulblets from th wheat by ordinary cleaning machinery.

THE TOTAL COST OF DRYING AND CLEANING GARLICKY WHEA

The total cost, including the shrinkage, of drying and cleaning a given lot of wheat for the removal of garlic depends on four facto (1) The amount of garlic removed; (2) the amount of chaff and oth reign substances, aside from the garlic, removed; (3) the percentage water removed from the wheat; (4) the cost of operating the chinery.

The amount of garlic removed.—In the experiments with the three s of wheat herein described practically all of the garlic was removed, d this must be considered as a loss in weight. The average loss each of the three lots of wheat due to the removal of garlic was 2 per cent, 0.50 per cent, and 1.88 per cent for Lots A, B, and C, pectively.

The amount of chaff and other foreign substances, aside from the garlic, noved.—The loss in weight due to the cleaning, aside from the quan- y of garlic, depends entirely upon the amount of light, immature eat, chaff, and other foreign substances removed. This loss bears e same ratio for any lot of wheat. Consequently, strictly speaking, is additional decrease in weight can not be considered as an extra pense in the treatment of garlicky wheat. Moreover, the quantity foreign substances present has an important bearing on the grading the grain.

The following summary shows the percentages of screenings, includ- the garlic, obtained from the wheat treated as experiments Nos. 4, 5, 6, and 7:

Experiment No. 3 gave 611 pounds of clean wheat and 28 pounds, 4.4 per cent, of screenings. Experiment No. 4 gave 548.5 pounds clean wheat and 15.5 pounds, or 2.8 per cent, of screenings. Exper- ent No. 5 gave 736.5 pounds of clean wheat and 26.5 pounds, or 3.4 r cent, of screenings. The average percentage of screenings from periments Nos. 3, 4, and 5 (Lot B) was 3.54 per cent. Deducting m this the amount of garlic removed from Lot B, 0.50 per cent, ere is left 3.03 per cent, the proportion of immature wheat, chaff, and er foreign substances removed.

In experiments Nos. 6 and 7, 1,536 pounds of dried wheat gave 135 unds of screenings, an equivalent of 8.8 per cent, of which 1.88 per t was garlic, leaving 6.92 per cent of immature wheat, chaff, and er foreign substances removed.

The percentage of water removed from the wheat.—Garlicky wheat ost invariably contains a high percentage of water, and the greatest s in weight is probably due to the liberation of water during the dry- process. In these experiments the quantity of water was reduced m 16.55 per cent, 15.08 per cent, and 16.20 per cent to an average 7.41 per cent, 7.76 per cent, and 8.52 per cent for Lots A, B, and C, pectively. In order that the garlic may be removed satisfactorily s necessary to reduce the water content to approximately 8 per cent, ich is from 4 to 6 per cent less than No. 2 Red wheat normally con- ns. However, the dried wheat will again absorb water from the osphere, and after the lapse of a few days the water content will be practically the same as that of air-dried wheat. Likewise the cl dried wheat can be mixed with any garlic-free lot of wet wheat and grade of the latter improved in this way. For this reason, only difference between the water content of the wet garlicky wheat that of No. 2 Red, which averages about 13 per cent during the f few months after harvesting, should be considered as actual lo weight due to drying. On this basis the loss due to the remov water was 3.55 per cent for Lot A, 2.08 per cent for Lot B, and per cent for Lot C.

The cost of operating the machinery.—The cost of the actual dr and cleaning alone is very small. With the low pressure boilers av able for use with the small grain drier in the Bureau of Plant Indu the maximum temperature possible is only 158° F. At this temp ture it is necessary to continue the drying for from two and one-ha three hours in order that the weight of the garlic may be suffici reduced so that it can be removed. With the high pressure boilers as are found in most grain elevators and flour mills an air tempera of 170° to 180° F. can be readily maintained, at which temperatur time factor can be greatly reduced. By careful calculation it is lieved that the actual cost of operating the machinery for the dr and cleaning should not exceed one-half cent per bushel.

This factor, however, will vary with the capacity of the drier the number and size of the other kinds of machinery being oper simultaneously by the same boilers.

THE NET COST OF REMOVING GARLIC.

To ascertain the net cost of removing garlic bulblets from whea order to bring it up to "contract" grade, only the following items be taken into consideration: (1) The cost of operating the machin (2) the loss in weight due to the quantity of garlic actually remo and (3) the difference in the amount of water normally containe good air-dried wheat, which is not far from 13 per cent, and the am of water in the garlicky wheat before it goes into the drier. On basis the cost of drying and cleaning the garlicky wheat discuss the foregoing pages was 6.3 per cent, 3.2 per cent, and 5.7 per cen an equivalent of 5½ cents, 2¾ cents, and 4⅘ cents per bushel for A, B, and C, respectively, as governed by the prices current at time.

THE EFFECT OF THE DRYING ON THE MILLING QUALITIE THE GRAIN.

No flour was made from any of the wheat after drying and clea but the consensus of opinion of the majority of the millers to w samples of the dried wheat were submitted was that the milling q ties of the wheat had not been injured by the drying. Such wh wever, is not fit for milling until it has absorbed water from the nosphere, or has been mixed with damp grain, or steamed, in order toughen the bran. If the milling is attempted while the wheat exceptionally dry, the bran will be easily broken, resulting in the duction of coarse, dark flour.

E EFFECT OF THE DRYING ON THE VITALITY OF THE WHEAT.

The objection has frequently been made that the high temperature linarily used in the commercial drying of grain will destroy the minating power. In the majority of cases the vitality of the grain er drying is of little importance, as such grain is seldom used for ring or planting. The foregoing objections, however, are not well ablished, as the vitality of grain is not injured by drying in com-ercial grain driers at the temperatures commonly employed.

The grounds for the belief that temperatures as high as 140° to 175° for periods of short duration will destroy the vitality of grains are sed on laboratory tests in which no provision was made for the cir-ation of air. Under such conditions the life-giving principles are dily destroyed, especially when considerable moisture is present. it when the drying is done in such a way that the moisture liberated l be readily carried away, as in commercial grain driers, there is le danger of destroying the vitality of the grains, even though the ration of drying be several times greater than that given for the egoing experiments.

The following table shows the effect of the drying on the germinat- capacity of the samples of garlicky wheat from lots B and C, eady discussed:

Percentages of germination of wheat from lots B and C before and after drying.

Sample mark.	Duration of drying.	Temperature of air current in drying.	Maximum temperature of wheat.	Water content of wheat.	Germination.
	Hours.	*Degrees F.*	*Degrees F.*	*Per cent.*	*Per cent.*
inal sample				15.08	80
riment No. 3	3	153–158	155	7.92	83.5
riment No. 4	3½	153–158	145	6.88	85
riment No. 5	2¾	155–108	138	8.48	79.5
inal sample				16.20	82
riment No. 6	2½	113–154	149	8.20	83
riment No. 7	3	146–122	131	8.83	85

With but a single exception the percentages of germination were her after the drying than before, and such is generally true. In cases the germination was low, due to the damaged condition of the in when received.

While the tests made are few in number, the results given in the going table are sufficient to show that a good quality of garlicky eat can be dried and afterwards cleaned and used for sowing with irely satisfactory results. The garlic bulblets, as found in wheat, contain from 35 to 70 per cent of water. With this high percenta of water the greater quantity of the bulblets are partially cooked scalded during the drying process, thus rendering growth impossib

MACHINERY USED FOR DRYING AND CLEANING.

The drying was done in a small grain drier. In the absence of good fanning and screening machine for the larger grains, the whe was first run through a fanning mill specially constructed for cleani clovers, alfalfa, and timothy. The greater quantity of the garlic w blown out, but many of the larger bulblets could not be removed the absence of screens, and for this reason the wheat, for the fin cleaning, was put through a "shaker" such as is commonly used f cleaning rice.

It is not desired to place any special emphasis on the particul machinery used for these experiments. Any of the good commerc driers with any good cleaning machinery should give satisfacto results.

SUMMARY.

The presence of the aerial bulblets of wild garlic in a large quant of the wheat grown in the central eastern United States causes a gr depreciation in its value. The loss to agriculture from this ca alone is very conservatively estimated at more than $1,500,000 ann ally.

The wheat kernels and the garlic bulblets are very similar in s and weight, which makes their separation by the methods ordina in use next to impossible as long as they are fresh.

If wheat containing garlic is artificially dried, the wheat kern increase in specific gravity and the garlic bulblets decrease in speci gravity, so that practically all of the latter can be removed by go cleaning machinery.

Garlicky wheat is usually wet, often containing as much as 20 p cent of water, and the drying should be continued until the moistu is reduced to approximately 8 per cent.

In estimating the total cost of the treatment of a lot of garli wheat, only the amount of garlic removed, the excess of moistu above that which good No. 2 Red wheat usually contains, and t cost of operating the machinery need be considered. The cost removing the chaff, immature wheat, etc., is the same as for the cle ing of any sample of wheat free from garlic.

The commercial drying of wheat in a good commercial grain dr does not injure its vitality, while most of the garlic bulblets are kill owing to the higher percentage of water in the latter.

It has not been definitely determined, but the more general opini is that the drying does not injure the milling qualities of the wheat.

Any of the good commercial grain driers, together with any go wheat-cleaning machinery, should give satisfactory results.

I.—212.

IV.—METHODS OF TESTING THE BURNING QUALITY OF CIGAR TOBACCO.[a]

By WIGHTMAN W. GARNER, *Scientific Assistant, Plant Breeding Investigations.*

INTRODUCTION.

.s has been pointed out in previous publications of the Bureau of
at Industry, a systematic effort is being made to improve the qual-
and yield of the tobacco crop by employing the latest and most
roved methods of selection in the old varieties and by creating
establishing new strains possessing to a marked degree those
:acteristics most to be desired in the various classes of tobacco
ch the market demands. This work necessitates the careful test-
of a large number of types, as well as many individual selections
n each of these types, and for this reason it is very desirable to
at our command methods capable of showing with certainty even
it differences in the essential qualities of the various samples to be
nined. It is our purpose to make a careful study of the subject
esting tobacco from a practical standpoint, as well as the relation
ie chemical composition of the leaf to its good and bad qualities.
judging the merits of a cigar tobacco, due regard must be had
the particular use for which it is intended, since the finished cigar
ists of three distinct components—the filler, the binder, and the
pper—each of which must possess certain characteristics. The

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n the tobacco breeding experiments conducted by the Plant Breeding Investiga-
of the Bureau of Plant Industry, particular attention is being given to the
ovement of cigar tobaccos, including specially high-grade wrapper and filler
. In connection with these experiments, which are being conducted by Messrs.
Shamel and W. W. Cobey, of this office, it has been found necessary to com-
the characters of a large number of selected individual plants to determine
h ones are superior in their important characters. The means and methods
ofore used in making such comparative tests were very imperfect, and one
rtant preliminary part of the work is to devise special pieces of apparatus which
enable accurate tests to be made. The devices described by Dr. Garner in the
nt paper it is believed will greatly facilitate such testing and add to the accuracy
e results. The preliminary notes given by Dr. Garner on the influence of
per, binder, and filler on the "burn" of cigars open up an important field of
tigation in connection with the testing and breeding of different types of
co.—HERBERT J. WEBBER, *Physiologist in Charge of Plant Breeding Investigations.*

filler must have, above all else, a fine flavor and aroma and a go "burn." In the case of the wrapper leaf there are a number requirements to be met, among which are sufficient elasticity, pro color, size, and shape, fineness of veins, freedom from objectiona flavor and taste, a fine "grain," and a good burn. Many of th qualities can be determined by simple inspection, without the use any specific tests, while others require special laboratory metho The present article has to do only with the practical methods of testi the burn, deferring to a later day a consideration of the chemic characteristics of the tobaccos which have been tested.

There are several elements which go to make a good or bad bu chief of which are the capacity for holding fire, the evenness of t burn, the color of the ash and its firmness, the coaling or carboni tion, and the "puckering" of the leaf immediately in advance of t burning zone of the cigar. The final test of any cigar tobacco mu of course, rest in the smoking of the manufactured cigar, but, wh this gives a direct means of determining the character of the ash does not furnish accurate information as to the evenness of the bu or the fire-holding capacity of any one of the components of the cig except with reference to the other two particular components used the experiment. This is particularly true of the wrapper, as w shown by special experiments carried out to observe the effect using different fillers and binders with the same wrapper. The res of these experiments will be more fully discussed below. Again should be remembered, in this connection, that cigars made by same workman and from the same lot of tobacco often vary widely their burn owing to the impossibility of avoiding unevenness in filler, and this source of error can only be eliminated by several tim repeating the experiment. It is evident, therefore, that, in order get reliable data concerning the relative merits of different wrapp with respect to their burning qualities, the cigar test must be supp mented by some other method capable of giving sharp distinctions as the fire-holding capacity and evenness of burn.

A method which has long been in use is to ignite the leaf by me of a lighted cigar or a slow-burning match devised by Nessler,[a] note the number of seconds during which it continues to glow. mean of several tests is taken as a measure of the capacity for holdi fire; but the variation in the results obtained, even upon a single le is so great that little reliance can be placed upon the figures except a very general way. In this method no account is taken of the a of the leaf burned, and the wide differences obtained on a single l are due principally to the fact that frequently the ignited zone so ceases to glow except for one or more very small streamers, whi

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[a] Landw. Vers. Stat., XI, 399.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 100.

B. T. GALLOWAY, *Chief of Bureau.*

ISCELLANEOUS PAPERS.

I. CRANBERRY SPRAYING EXPERIMENTS IN 1905.

By C. L. SHEAR, *Pathologist.*

II. THE WRAPPING OF APPLE GRAFTS AND ITS RELATION TO THE CROWN-GALL DISEASE.

By HERMANN VON SCHRENK, *Special Agent,*
and GEORGE G. HEDGCOCK, *Assistant.*

III. GARLICKY WHEAT.

By J. W. T. DUVEL, *Assistant.*

V. METHODS OF TESTING THE BURNING QUALITY OF CIGAR TOBACCO.

By WIGHTMAN W. GARNER, *Scientific Assistant.*

V. THE DRUG KNOWN AS PINKROOT.

By W. W. STOCKBERGER, *Expert.*

VI. ORCHARD GRASS.

By R. A. OAKLEY, *Assistant Agriculturist.*

VII. THE EFFECT OF COPPER UPON WATER BACTERIA.

By KARL F. KELLERMAN, *Physiologist,*
and T. D. BECKWITH, *Scientific Assistant.*

VIII. CONDITIONS AFFECTING LEGUME INOCULATION.

By KARL F. KELLERMAN, *Physiologist,*
and T. R. ROBINSON, *Assistant Physiologist.*

ISSUED APRIL 25, 1907.

check valve consisting of a T-tube (e), the lower arm of which dip beneath the surface of the water in a suitable vessel (f), thus prevent ing a backward draft through the cigars while the aspirator is filling The smoke which escapes through this valve has a very disagreeable odor; hence, it is well to use a bottle fitted with a stopper with two holes, through one of which passes the lower arm of the T-tube (g), while through the other passes one end of a long tube (h), which carries away the obnoxious fumes. The aspirator consists of a glass cylinder (i), the upper end of which is fitted with a rubber stopper

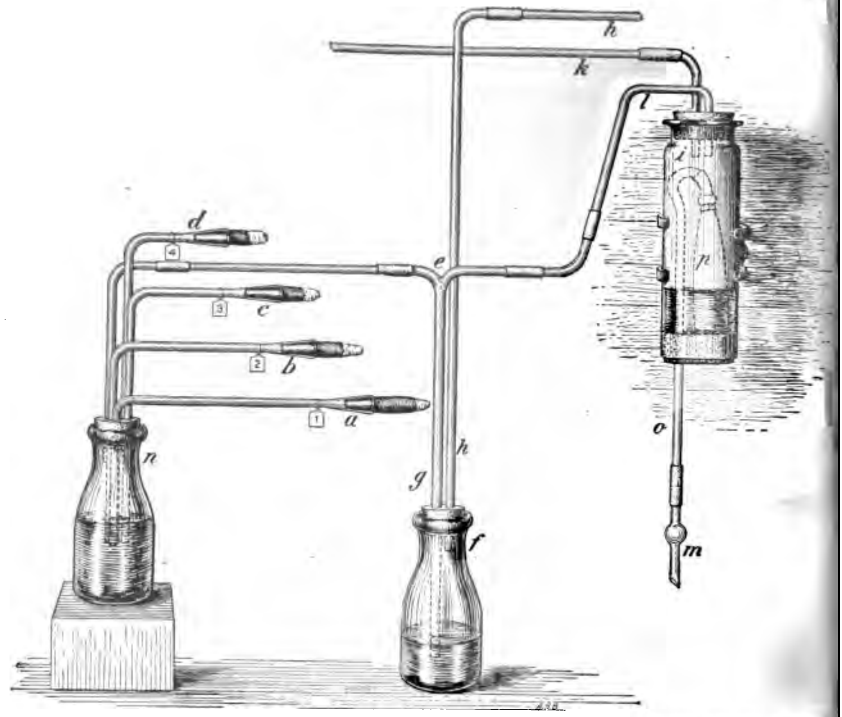
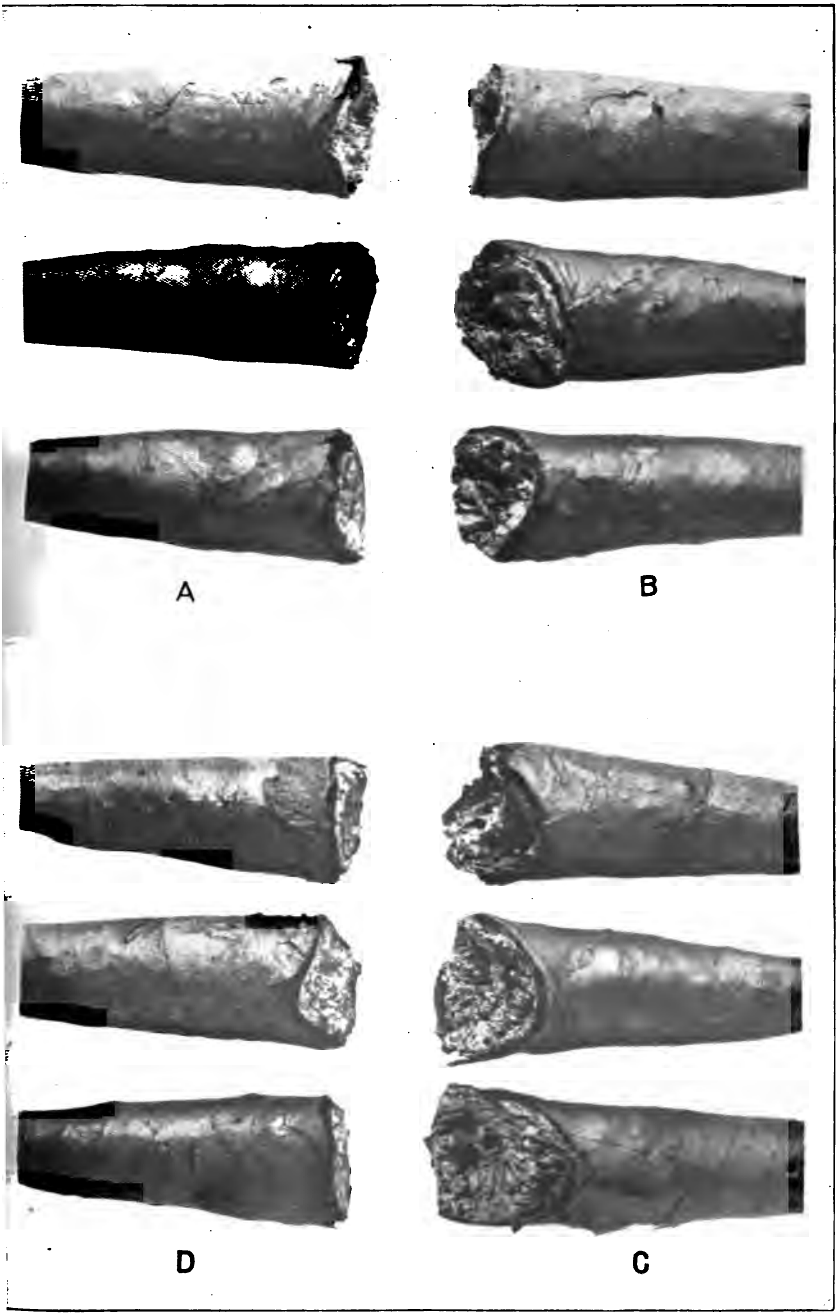


FIG. 2.—Apparatus for testing the burning quality of cigars: a, b, c, d, holders for cigars; e, T-tub the lower arm of which, g, dips beneath surface of water in f; h, h, tube for leading away tobac smoke; i, aspirator; k, tube leading to water supply; o, p, long and short arms of siphon; l, tu connecting cigar holders with aspirator; m, tube, with bulb, attached to long arm of siphon; flask carrying the cigar holders.

containing two holes through which pass tubes leading to the water sup ply (k) and to the flask carrying the cigar holders (l), respectivel The lower end of the cylinder is closed with a rubber stopper bearin the small arm of the siphon (o). The tube furnishing the water sup ply is connected with a reservoir provided with a constant-level attach ment. To the lower end of the small arm of the siphon is attached glass tube (m) bearing a small bulb which materially assists in breal ing the flow of the water at the moment the aspirator is emptied. Th tubing connecting the parts of the apparatus should not be less tha



VARIATION IN BURN OF WRAPPERS DUE TO DIFFERENT FILLERS: A, FILLER GROWN IN TEXAS FROM CUBAN SEED; B, FILLER GROWN IN OHIO FROM DOMESTIC SEED; C, FILLER GROWN IN SOUTH CAROLINA FROM CUBAN SEED; D, AN IMPORTED CUBAN FILLER.

The same sample of Sumatra wrapper was used throughout and the binder was taken from the same leaf as the wrapper in each case.

to 8 mm. internal diameter; otherwise the tubes will frequently become logged by the condensation products of the smoke. The container or the cigar holders (*n*) is filled about two-thirds full with dilute sulhuric acid, which serves as an acid wash for the smoke, retaining the rganic bases and thereby further helping to prevent the choking up f the machine. The lower ends of all the cigar holders should, of urse, extend to exactly the same depth below the surface of the id.

In the machine which we now have in operation, the relation between e short arm of the siphon and the internal diameter of the aspirator such that the volume of water delivered at each emptying of the tter is 600 c.c. This corresponds to an actual capacity of about 450 c. for the aspirator, the difference, of course, representing the volume f water entering the aspirator from the supply pipe while the siphon in action. The rate of inflow from the supply tank is approximately 0 c. c. per minute. The internal diameter of the long arm of the phon is 8 mm., while that of the short arm is 25 mm. The entire ngth of the long arm of the siphon exceeds that of the short arm by cm. An apparatus of the above-mentioned dimensions will smoke ur cigars of the Perfecto type, 4⅞ inches in length, in about thirty nutes, a rate which is probably somewhat above that of the average oker. The pull on the cigar occurs at intervals of thirty seconds d continues for a period of ten seconds. The frequency of the pull ontrolled by the rate of inflow of the water from the supply tank, ile its duration is governed principally by the relation between the meter of the small arm of the siphon and the volume of the irator.

E EFFECTS OF THE FILLER, THE BINDER, AND THE WRAPPER ON THE BURN OF THE CIGAR.

s preliminary to the use of the cigar test in examining wrapper f, a series of experiments was carried out to determine the relative ects of the three components of the cigar on the burn. For this pose a number of cigars were made by an expert workman, using r different types of wrapper on each of four different types of r. In a portion of the cigars the binder used was taken from the e leaf as the wrapper, while in the remainder a sample of Connecti- Broadleaf tobacco was employed for this purpose. These cigars e smoked in the above-described apparatus under conditions as rly uniform as could be obtained, and the evenness of the burn and character of the ash were carefully noted.

ith reference to the evenness of the burn, markedly different lts were obtained when wrappers taken from the same sample were ked on different types of filler. A typical case of this kind is shown late III. The twelve cigars shown were all made from the same sample of wrapper, and in each case the binder was taken from t same leaf as the wrapper. In the first group (A), a sample of fil grown in Texas from Cuban seed was used; in the second group (B), heavy filler grown in Ohio from domestic seed; in the third group (C a filler grown in South Carolina from Cuban seed; in the fourth gro (D), an imported Cuban filler. The wrapper used on these cigars w a type of Sumatra tobacco grown in Connecticut and had a very go burn. The Texas and imported Cuban fillers were known to have excellent burn, while the South Carolina filler was markedly infer in this respect and the Ohio filler intermediate in burning qualities. will be seen that this sample of wrapper burned quite evenly wh used with the imported Cuban and Texas fillers, while with the Oh and especially the South Carolina fillers the burn was decidedly unev

On the other hand, the effect of using different types of wrapper the evenness of burn of any one type of filler was less marked (see IV, A, B, and C; also Pl. III, D). The filler used in this experim was the imported Cuban, while the wrappers were taken from four d ferent types of Sumatra tobacco grown in Connecticut. Of these fo types of wrappers, that shown in Plate III, group D, had the best bu and the one shown in Plate IV, group C, the poorest, although li difference could be seen between the two when smoked on the Cub filler. The use of different binders did not cause any marked dif ences in the evenness of the burn, as is shown in Plate IV, group and D. The cigars used in this experiment were all made from same wrapper and the same filler, while in group D a sample of C necticut Broadleaf tobacco was used as the binder, and in group C binder was taken from the same leaf as the wrapper.

Another important factor in determining the evenness of the bur the proper balancing of the component parts of the cigar. It found, for example, that a very light[a] wrapper will not give g results on a heavy filler, even though both of these may in them possess a good burn. It will readily be seen that a very thin wrap which burns readily and very rapidly will, when placed on a hea slow-burning filler, tend to burn in advance of the latter, and the ef will generally be an uneven burn. The same result is obtained w any cigar is smoked very rapidly, for the reason that the oxyge the air has freer access to the outer edges of the burning zone under the added stimulus it rarely happens that a cigar will b evenly.

As regards the character of the ash, the wrapper and the binder relatively of much more significance. It was found, it is true, some fillers give an ash lacking in compactness and liable to sp asunder, but the tendency to flake seems to be controlled almost entir

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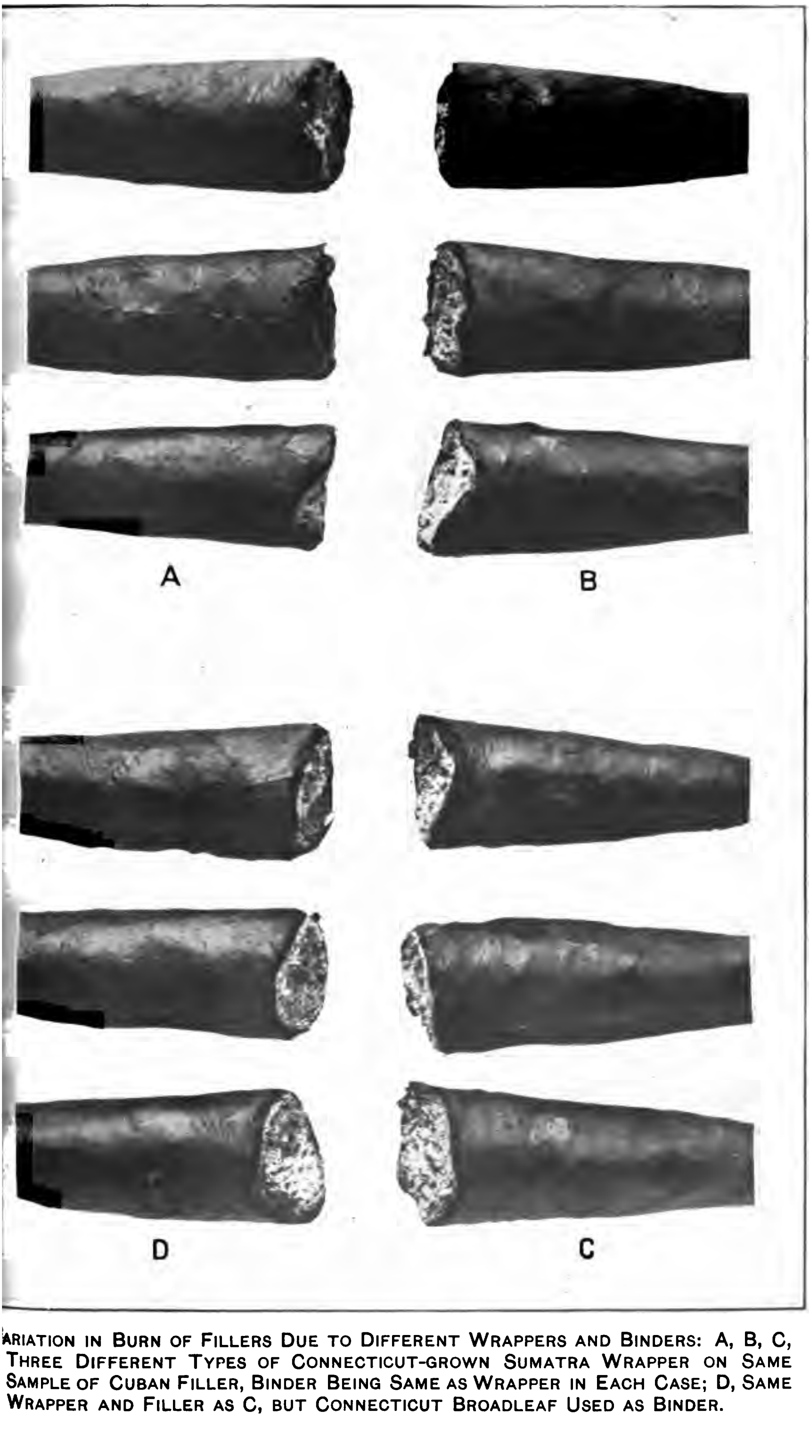
[a] The terms *light* and *heavy* as used in this connection refer to the body or thi ness of the leaf, which largely controls the rapidity of the burn.



ARIATION IN BURN OF FILLERS DUE TO DIFFERENT WRAPPERS AND BINDERS: A, B, C, THREE DIFFERENT TYPES OF CONNECTICUT-GROWN SUMATRA WRAPPER ON SAME SAMPLE OF CUBAN FILLER, BINDER BEING SAME AS WRAPPER IN EACH CASE; D, SAME WRAPPER AND FILLER AS C, BUT CONNECTICUT BROADLEAF USED AS BINDER.

y the character of the wrapper and binder. Furthermore, this lack cohesion in the ash of a wrapper may be largely overcome by the e of a good binder. As to the color of the ash, a binder having a od burn will impart to the ash of the wrapper a lighter tint and a ore uniform color. The general results of these experiments may summarized as follows:

(1) In order to secure a good burn, due consideration should be given the proper balancing of the components of the cigar; that is, a heavy ler should be wrapped with a comparatively heavy wrapper, while a ht-bodied filler requires a light-bodied wrapper.

(2) Of the three components of the cigar, the filler exerts the strongest fluence on the evenness of the burn.

(3) The influence of the wrapper and binder is shown most strongly the character of the ash, and the binder very materially influences e ash of the wrapper in this respect.

TESTING THE CAPACITY FOR HOLDING FIRE AND THE EVENNESS OF THE BURN.

The factors of holding fire and of burning evenly are of prime portance in judging the burn of tobacco, and any sample found rkedly deficient in these points may be rejected without applying y further test. As has been previously stated, the old method of asuring the fire-holding capacity is likely to lead to erroneous con- sions, while there has heretofore been no direct method of deter- ning the evenness of the burn of wrapper leaf. In the process ich we have used for testing wrapper tobacco with regard to these ments of the burn, the area of the leaf consumed, rather than the e elapsing before the glow is extinguished, is measured.

The form of the apparatus used in this method will be understood reference to the accompanying illustration (fig. 3). The essential ture is the form on which the leaf is wrapped, consisting of a col- sible wooden tube, one end of which fits into a glass tube of the ne diameter. This latter is in turn connected with a second glass e through which is drawn a current of air. The best material for king the wooden form is well-seasoned cherry with a straight grain, t ash has also been found to answer the purpose very well. From the od selected is made a cylinder 5 inches in length, ¾ inch in diameter one end, and tapering slightly to the other end (see fig. 4). In the ger end of the cylinder a ⅝-inch hole is bored to a depth of 3¾ inches, l the shell thus formed is separated into six equal segments by saw- ; to a depth of 3½ inches. The smaller end is cut down for a dis- ice of 1½ inches, so as to fit snugly into the glass tube. The shoulder is formed should correspond in depth to the thickness of the wall of glass tube. Near the larger end of the form a groove (*c*) is cut, o which is fitted a rubber band. The plug (*d*) has a diameter such that when inserted in the end of the form the latter is expanded to its original size. The receiver (*a*) for the form is made by drawing out one end of a short piece of thick-walled glass tubing. All of the abov dimensions are based on tubing having an internal diameter of 14 m (9/16 inch) and an external diameter of 18 mm. (11/16 inch). Th small end of the receiver is fitted with a soft cork (*b*), by means of which it is connected with the other portion of the apparatus.

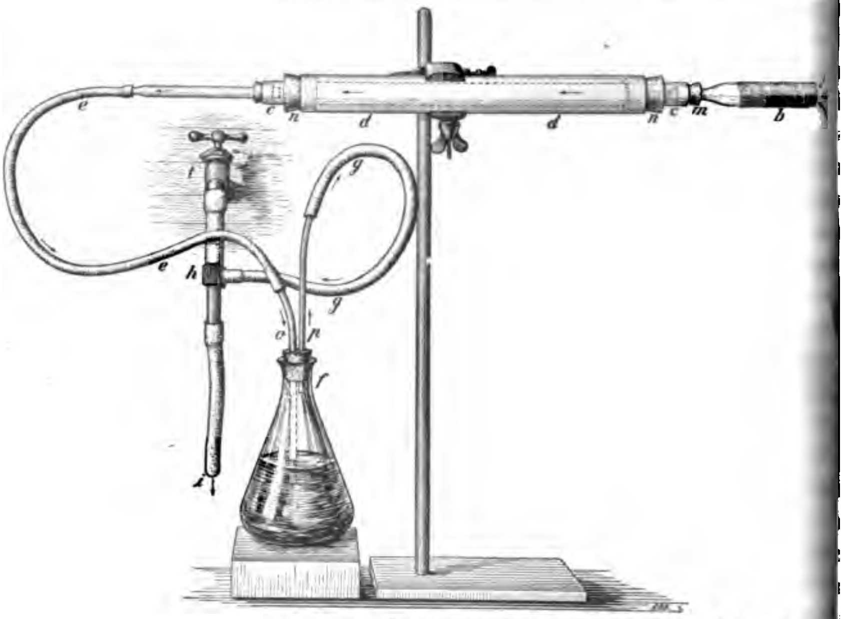


Fig. 3.—Apparatus for testing the burning quality of wrapper tobacco: *a*, entrance of air current; wrapper to be tested; *c*, *c*, glass tube to which the form bearing the wrapper is attached by me of the cork, *m*; *d*, *d*, large glass tube fitted with corks, *n*, *n*, through which passes *c*, *c*; *f*, fl containing water; *o*, small glass tube dipping beneath the surface of the water in *f*; *p*, short gla tube leading from *f*; *h*, pump by means of which the current of air is secured; *e*, *e* and *g*, *g*, rubb tubing connecting parts of apparatus; *t*, water tap; *i*, outflow of water.

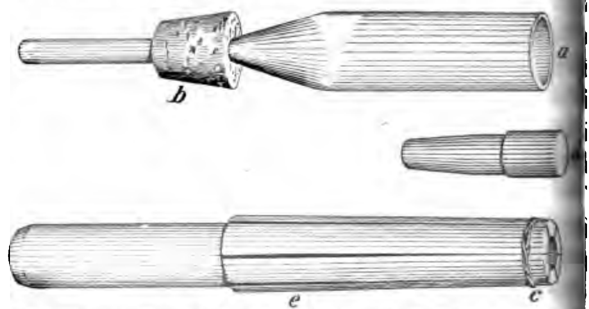


Fig. 4.—Construction of form on which leaf is wrapped for use apparatus shown in figure 3: *a*, glass tube for receiving the form cork by which receiver (*a*) is connected with remainder of appara shown in figure 3; *c*, rubber band for collapsing the form; *d*, p for expanding the form; *e*, form on which leaf is wrapped.

From the leaf to be tested, which should be quite damp, the wrapper is cut into a form quite similar to that used for cigars, and the same rules are observed as regards the cutting of right-handed an left-handed wrappers, etc. Beginning at the outer end the wrapper lled quite tightly, first on the form and then on the glass. At the ginning of the process of rolling, the extreme outer corner of the se of the wrapper is attached to the overlapping portion with a bit cigar paste, and at the end of the operation the tip of the wrapper attached to the receiver by the same means. A number of samples be tested are thus wrapped on the forms and set aside until they ve dried out properly. The plug in the end of the form is then ithdrawn and the rubber band causes the walls of the latter to col- pse, so that it can be easily withdrawn from the receiver. This aves the sample of wrapper securely attached to the glass tube, d in exactly the same form it would have on a cigar. The tube rrying the sample to be tested is connected with the remainder of e apparatus, shown in figure 2, the construction of which will be derstood without further explanation. The current of air is fur- shed by means of an ordinary filter pump, and its rate can be con- olled with sufficient accuracy by measuring the flow of water through e pump. The end of the wrapper is ignited with a flat gas flame, d the evenness of the burn and the portion consumed before it ceases glow are carefully noted. Our method of recording the results is grade each sample on a scale of ten, both with reference to the enness of the burn and the fire-holding capacity. Of course, stand- ds in these tests are purely arbitrary, as the results are only intended be comparative. Under the conditions laid down for the experi- nt, wrappers having markedly good burning qualities will burn up mpletely and evenly with only one lighting, and these are given a ade of 10.

For the purpose of comparing the results obtained by this method th those given by the cigar test with reference to the evenness of the rn, a number of leaves were selected from different types of wrap- r tobacco. One half of each leaf was used for wrapping a cigar and e second half was wrapped on the form for testing, as has just been scribed. There was a decided lack of agreement in the results tained by the two methods when only one type of filler was used in king the cigars. It was found that frequently a wrapper that aded only 5 or 6 on a scale of 10 in what may be called the "form st" would burn quite evenly on the cigar, whereas another wrapper ading as high as 9 in this test would show an uneven burn on the gar. A good illustration of this point is found in a wrapper which s scored 10, 9, 10, respectively, in three experiments with the form st and gave a fire-holding capacity of 65 seconds by the old method Nessler. On one type of filler this wrapper gave a very uneven rn, but when smoked on a lighter filler the burn was perfectly satis- ctory. These results, then, seem to emphasize the fact that, although e final judgment as to the burning qualities of a wrapper which has own up well in the preliminary tests must be based on the smoking of the cigar, great care must be exercised to avoid the sources of error in this test which have been previously discussed. The test should not only be repeated with a single type of filler to avoid the effects of any possible unevenness or other imperfections in the manufacture of the cigars, but at least two different types of filler should be used, one of these being heavy and the other light in the sense in which these terms are used here.

TESTING THE BURN OF CIGAR-FILLER TOBACCO.

Testing the burn of a filler is a much simpler problem than is the case with a wrapper. The principal elements of the burn are the evenness and the capacity for holding fire, and the character of the ash is unimportant, except that it should be compact. The evenness o the burn and the fire-holding capacity are best determined by usin the cigar test. In the case of filler tobacco the capacity for holdin fire thus refers simply to the length of time the cigar will continue t burn after being lighted without being puffed by the smoker. Th effects of the binder and wrapper on the burn may be avoided b making the entire cigar from the filler leaf to be tested. Anothe decided advantage in making the whole cigar from the same tobacco is that the aroma, which is so important in the filler, can also be teste at the same time. In determining the fire-holding capacity it is only necessary to light the cigar and test it at gradually increasing interval of time to find whether it has ceased to burn. It is, however, desir able to test the fire-holding capacity and the evenness of the burn on separate cigars if sufficient material is at hand for this purpose.

I.P. I.—229.

V.—THE DRUG KNOWN AS PINKROOT.

By W. W. STOCKBERGER, *Expert, Drug-Plant Investigations.*

INTRODUCTION.

The drug known as pinkroot is derived from the underground portions of *Spigelia marilandica* L. (Pl. V), an American herb now found growing most abundantly in the Southern States and occurring locally in the Mississippi Valley and eastward. It came into use in America as a vermifuge about 1723 and because of its valuable properties soon came to occupy an important place in materia medica. Unfortunately, however, conflicting reports on its physiological effects in time established for pinkroot a reputation for uncertain action, and within the last fifty years the use of this drug, once regarded as highly reliable and valuable, has greatly decreased. Since it has seldom been held at high prices the cost has not operated to drive it out of the markets.

The cause of the apparent loss of high efficiency formerly claimed for pinkroot has engaged the attention of students of crude drugs for many years. The demonstration by Dr. R. H. True[a] that an unsuspected substitute had crept into the markets and to a considerable degree replaced the true article has explained in large measure the unfavorable commercial and medical status of pinkroot. The results here outlined of a detailed study of pinkroot and its more important adulterants may serve to aid collectors in discerning the real pinkroot and to assist drug experts in distinguishing the plant from its sophistications in its commercial form.

TRADE VARIETIES OF PINKROOT.

The complex nature of the material put upon the market as pinkroot has long been known to the drug trade, and although the real nature of the spurious article was not understood, its presence was recognized, and various sorts of pinkroot came to be distinguished by definite trade names—e. g., true pinkroot, genuine pinkroot, southern pinkroot, Georgia pinkroot, East Tennessee pinkroot, western pinkroot, and true fiber pinkroot. The visible differences by which these trade varieties are segregated may be utilized in distinguishing the true from the false pinkroot, as it is now definitely known th certain of the trade varieties are wholly composed of worthless sub stitutes.

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[a] Pharmaceutical Review, 21: 364, 1903.

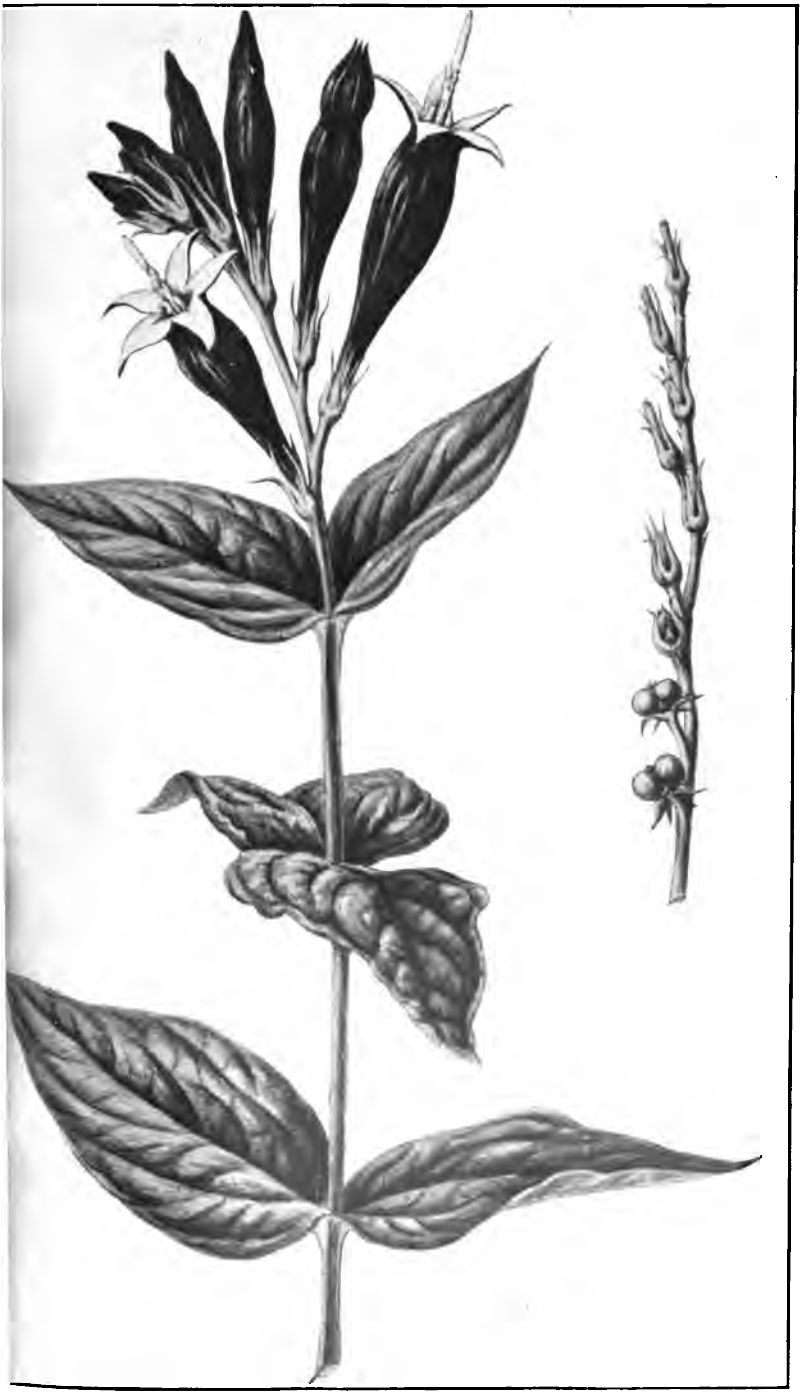
In many cases careless or unscrupulous collectors and dealers hav not regarded the distinguishing features of the various sorts of pink root, or have been ignorant of them, with the result that very gener confusion exists as to the character of the real drug and .its adulter ants. The authors of some prominent publications on crude drug have evidently based their observations on trade varieties of pinkroo as they have illustrated and described one of the most important adulter ants as true pinkroot.

IDENTITY OF CHIEF SUBSTITUTES.

East Tennessee pinkroot (*Ruellia ciliosa* Pursh), a member of t plant family Acanthaceae, is the most important adulterant. Obser tions on the plant structures present in commercial samples of pinkr convinced Dr. R. H. True, in 1900, that the chief part of this cru drug consisted of a substitute instead of Spigelia and that this subs tute was a species of Ruellia. In trying to get plants of pinkroot f cultivation, Doctor True, in 1903, purchased several hundred ro from a dealer in eastern Tennessee. These roots were set out in t testing gardens of the Bureau of Plant Industry at Washington, D. C and the plants kept under close observation. On developing th were found to differ markedly from Spigelia, and upon flowering we identified as *Ruellia ciliosa* (Pl. VI), a plant which had never appeare in the list of suspected adulterants prior to this time.

The examination of the microscopic structure of this plant recall at once the figures in some text-books purporting to represent Spi lia and those illustrating an article by Greenish in the Pharmaceuti Journal, 1891, on the structure of *Phlox carolina*, a plant which long been regarded as an extensive substitute for pinkroot. It evident that a double confusion existed with regard to Ruellia. the one hand it was so widely mistaken for Spigelia that its pecu structures have been regarded as diagnostic of pinkroot, and on other it was recognized as a substitute, but wrongly regarded as Phl a plant lacking many of the striking characteristics of Ruellia.

In order to satisfactorily determine the relation of these subst tions to the true pinkroot, observations have for three years b made upon plants of Spigelia, Ruellia, and Phlox under cultivatio Washington, D. C., and fresh material secured from them has b used in making a comparative study of their structure. The res of this study do not support the view that Phlox is an adulterant pinkroot, and, moreover, several samples of a substitution suppo to be Phlox have proved, upon examination, to be composed entir of Ruellia. It is only through long and familiar observation in



PINKROOT (SPIGELIA MARILANDICA L.).

ving condition of all species here concerned that it has been practi- ible to uncover fully the true relations involved in the drug called inkroot.

MINOR ADULTERANTS.

Aside from Ruellia, the adulterants of Spigelia may be regarded as npurities, due in the main either to the carelessness of the collector i not sorting out the roots with which the plant was associated in its rowth, or perhaps to a lack of familiarity with the plant on the part f young or inexperienced collectors. With Spigelia other roots ometimes occur which have a greater market value than the true inkroot, and therefore can not be regarded as intentional adulterants. he worthless roots frequently present, however, may have been ntroduced by the collector with full knowledge that a fraud was being erpetrated. In commercial samples of pinkroot, among other impuri- ies have been observed roots of golden seal (*Hydrastis canadensis* L.), erpentaria (*Aristolochia serpentaria* L.), soapwort (*Saponaria offici- alis* L.), wild yam (*Dioscorea villosa* L.), and stoneroot (*Collinsonia anadensis* L.).

METHODS OF DISTINGUISHING PINKROOT FROM ITS SUBSTITUTES.

Once familiar with the true pinkroot it is hard to see how any drug ollector could confuse it with the plants so frequently substituted in s place. Spigelia and Ruellia grow over large areas, largely over- pping and in much the same habitat, and have on the whole a cer- in general resemblance; but they should be readily distinguished by bserving any one of their several striking characteristics.

In Ruellia (Pl. VI) the flowers are borne scatteringly along the stem the axils of the leaves; in Spigelia (Pl. V) they are aggregated at e top of the plant in a one-sided spike. In Ruellia the pale magenta- lored corolla forms a slender tube below, expanded upward into a oad, flaring limb. The anthers and style are not protruded. In pigelia the corolla forms a rather broad tube, narrowest at the throat, olonged upward into spreading, narrow, triangular portions. The erior is brilliant cardinal in color, bright yellow on the inside; the le and anthers are exserted. The leaves of Ruellia are bright green, ally short-petioled or sessile, frequently more or less hairy. In gelia they are dark green, glossy, and sessile.

n the crude drug the forms are separated by less evident gross racters. Ruellia, however, has a coarser, harsher root system than gelia, and the roots show a tendency to lose the cortical tissues, ving the naked, woody cylinder exposed. The roots of Spigelia are icate, fibrous, and usually very numerous. When dry they break crumble very readily.

The differences in structure between Spigelia and its substitutions as seen under the microscope are usually very marked. This is especiall true of Ruellia, the only important adulterant found with Spigelia, since the other plant parts sometimes present are usually recognize by their gross characters. A comparison of figures 5 and 6 will sho the most important differences in minut structure. The numerous cystoliths presen in Ruellia as a very conspicuous feature ar wholly lacking in Spigelia and *Phlox ovata*. The large sclereids of the root of Ruellia ar not found in the other two plants, and starch which is presen in Spigelia, i absent in bot Ruellia and Phlox.

In the powdere form Ruellia always reveals it presence by th numerous ston cells and cystoliths, which usually remain intac even in finely powdered material. Powdered sample of the underground portions o the Phlox at han gave no reactio for starch. Th fineness of th starch grain o Spigelia and it lack of strikin characters rende uncertain its identification amon many other plant starches which might be readily introduced in th powdered drug. The starch grains of Spigelia measure about 4 μ and in powdered pinkroot are associated with parenchyma cells an long light-colored sclerenchyma fibers. The absence of starch from powder supposedly made of pinkroot suggests at once that the materi is not Spigelia. On the other hand, the presence of starch, whil indicative of Spigelia, is by no means conclusive proof of its presence.

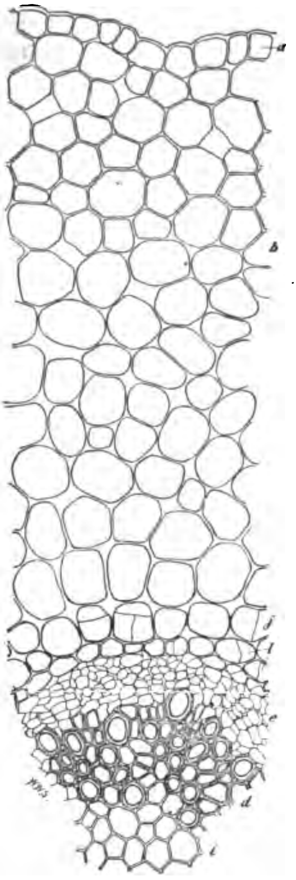


FIG. 5. Cross section of the root of *Spigelia marilandica* L.: *a*, epidermis; *b*, cortex; *d*, xylem; *e*, cambium; *i*, pith; *j*, endodermis; *l*, pericycle. × 180.

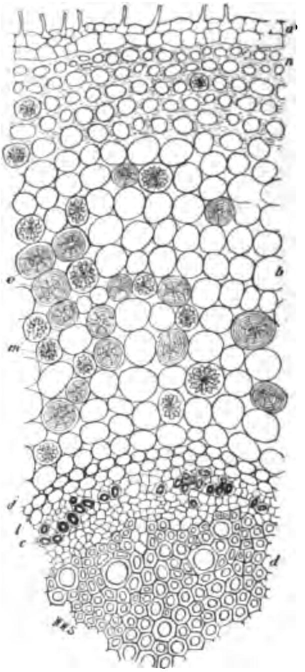
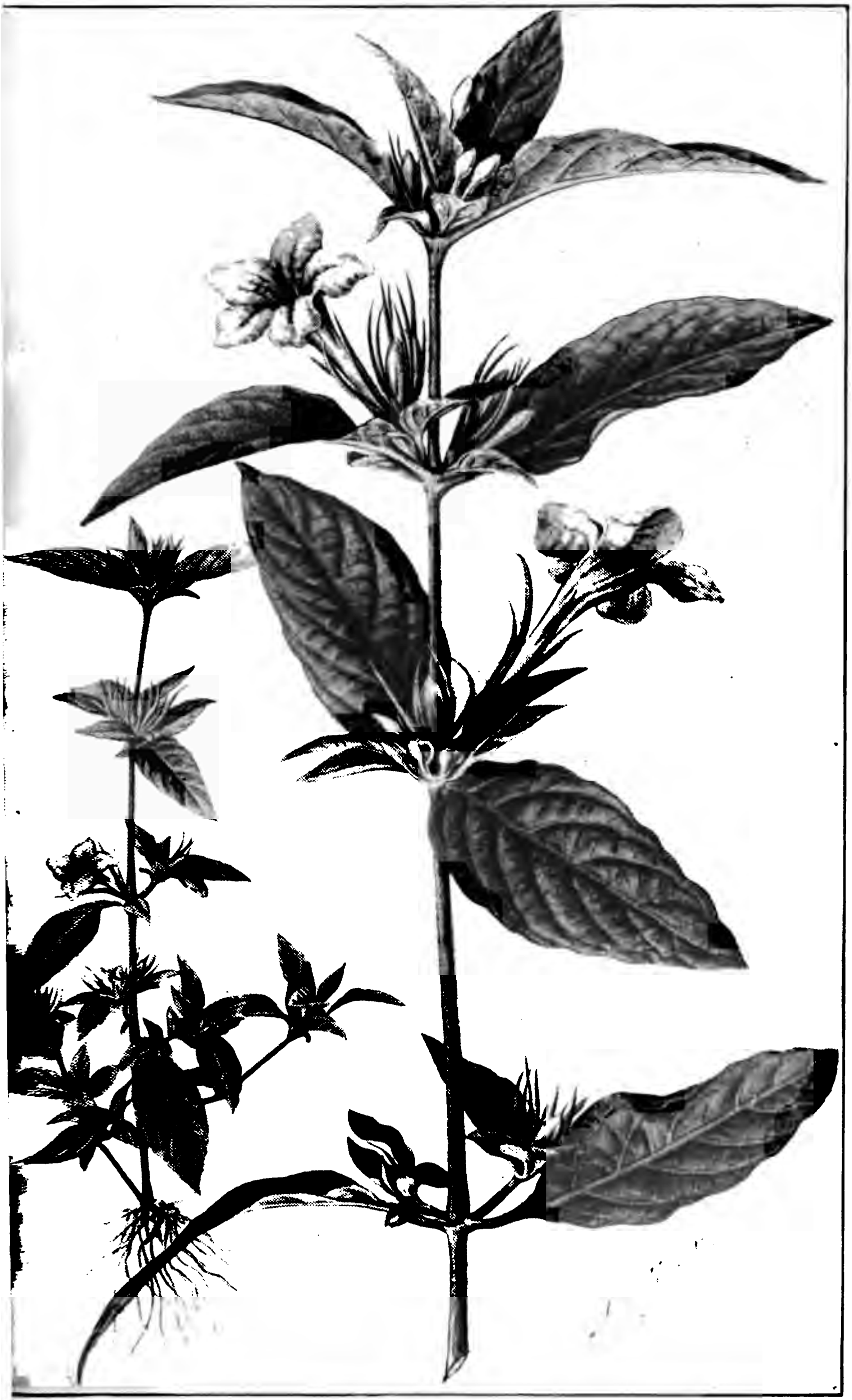


FIG. 6. Cross section of the root of *Ruellia ciliosa* Pursh : *a*, epidermis; *b*, cortex; *c*, bast fibers; *d*, xylem; *j*, endodermis; *l*, pericycle; *m*, cystoliths; *n*, collenchyma; *o*, sclereids. × 150.



EAST TENNESSEE PINKROOT (RUELLIA CILIOSA PURSH).

VI.—ORCHARD GRASS.[a]

By R. A. OAKLEY, *Assistant Agriculturist, Farm Management Investigations.*

INTRODUCTION.

Orchard grass (*Dactylis glomerata* L.) is a well-known standard rass which is grown to some extent in every State in the Union and uite commonly in the region east of the Mississippi River and north the northern portions of Alabama and Georgia. It attains most nportance, however, in Kentucky, southern Indiana, Tennessee, orth Carolina, Virginia, West Virginia, and Maryland, and seems uite thoroughly adapted to a variety of soils in these States.

It may be said that the general opinion of farmers in regard to the lue of orchard grass either for hay or pasture is quite unfavorable. his unfavorable opinion, which is due somewhat to prejudice, exists a greater extent in the timothy region than elsewhere, and as the nits of this region are reached and crossed orchard grass is much ore highly regarded. The objectionable features of the grass are in neral its bunchy habit, coarseness, and the unpalatability of its hay less cut at the proper state of maturity. These objectionable tures are not alone the cause of its unpopularity or the reason why is not grown more generally. There is no doubt that orchard grass uld be grown very successfully throughout the greater portion of e timothy region, but as the demand for any hay except timothy is ry limited farmers see little inducement for them to raise it. On account of its maturing well with a number of other very valuab grasses and clovers its popularity may in time increase as the adva tages of such mixtures become more generally appreciated.

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[a] In connection with the general plan of the Farm Management work it is contemted to take up the study of various seed crops. Much interest is now being manited in better seeds for the farmer. This is especially true of forage-crop seeds, luding both grasses and clovers. Mr. Oakley's paper, which is contributed from Office of Farm Management, conducted under the direction of Prof. W. J. Spilln, is a valuable contribution to the methods followed in growing orchard grass hay, for pasture, and for seed. Special attention is called to the fact that orchard ss seed as usually grown is for all practical purposes pure. The investigations of Bureau have shown that considerable quantities of the seed of this grass found the market contain seed of other and less desirable, cheaper grasses. That the ds of these cheaper forms have been added for the purpose of adulteration seems dent from the fact that the grasses bearing them are not found in orchard grass ds to any extent worth mentioning.—B. T. GALLOWAY, *Chief of the Bureau of nt Industry.*

Orchard grass is exceedingly variable and offers a large field f selection and breeding. Its variable characters of most importan are its coarseness, bunchiness, and time of maturing. By consiste selection with special reference to the first two characters valuab strains may in time be developed which will not possess the objectio able features of the common orchard grass now being grown.

METHODS OF CULTURE.

SEEDING.

While there are some methods that are generally employed in t culture of orchard grass, still there is a great difference of opini even among the most successful growers in any one locality as to t best practices. In the seed-producing section of Kentucky and Ind ana it is the common custom to sow the grass in February on f wheat at the rate of from three pecks to one bushel to the acre. Sin the crop in this section is almost entirely harvested .for seed, it is n considered desirable to sow more than a bushel. In years past much as two bushels to the acre were sown, but it is now the ge eral opinion that one bushel is sufficient, and even less is oft used. A bushel of orchard grass seed weighs 14 pounds. Orcha grass to give large yields of seed should be reasonably thin, it produces more abundantly when in this condition. It is usual sown broadcast, as it does not feed out well through a press dri either by hand or with a wheelbarrow or other type of seeder, and covered very shallow. Good results are often obtained by not cove ing the seed, and it is quite a common opinion that too deep coveri is the cause of many of the failures to secure a stand.

A method of seeding which is often followed in the section me tioned is to scatter the orchard grass straw from which the seed h been thrashed on ground that has been sown to wheat. This is usual done in February. The straw acts as a mulch in this case and the see needs no covering. It is very essential that it be scattered evenly an very thin; otherwise the stand will be too thick and unsatisfactor The greatest objection to this practice is that unless the straw is ve clean the meadow is sure to be weedy, and some are of the opi ion that since the seed that is left in the straw or blown over with is mostly of poor quality a field of inferior and unequally maturi grass will be the result.

Orchard grass may be sown successfully after corn by splitting t rows with a disk harrow as soon as the crop is removed. This ma be done any time during the month of October, and in February or n as the weather is favorable the grass may be sown with a broad- seeder at the ordinary rate, and the ground being uneven at this e the freezing and thawing which follow will cover the seed suffi- tly. Disking seems to give much better results than plowing, e the ground if plowed will not have time to thoroughly settle re the time of seeding. Rolling would doubtless be beneficial r seeding in this manner.

n western Virginia and in Tennessee orchard grass is commonly n the latter part of September or the first of October with wheat the ground at the rate of a bushel and a half to two bushels to the e, the wheat being drilled in at the rate of three to five pecks to the e and the grass sown either broadcast by hand, with a broadcast er, or an attachment to the drill, and covered as shallow as possible.

rchard grass is often sown with oats, usually in March, on ground has been previously in wheat and which has been plowed the pre- ng autumn. A half seeding of oats is usually sown in this case, gives as a result only a fair crop. The grass, however, makes e pasture the first season as a rule than when sown with a full ing of either wheat or oats. Early fall seeding with winter oats ections where the latter can be grown may be depended upon to good results, but on account of the Hessian fly it is not possible ow it with wheat much before October.

crop of hay is not expected the first season, whether the grass is n in the fall or spring, either alone or with a nurse crop. If sown e a light cutting may be secured, provided the conditions are orable, in the latter part of August or September; but in general grass is pastured and not cut except at the time when the grain ch is sown with it is harvested. The following season it makes a p of either hay or seed, as is desired. There may be some advan- e in sowing the grass alone for the extra quantity of forage pro- ed the first year, but whether this and any other advantages that y come from seeding in this way will compensate for the profit uing from the nurse crop is an undecided question.

MIXTURES WITH RED CLOVER.

Throughout almost the entire region where orchard grass is grown s quite a common practice to sow red clover with it. This practice good one, not only for the value of the red clover in maintaining soil fertility, but also for the fact that its presence greatly improves orchard grass either for hay or pasture. In the seed-producing tions red clover is a menace to the seed crop, especially the first and ond years. As it is impossible to cut the orchard grass above the ver, the leaves and heads get mixed in with the seed and are difficult separate from it. Although the presence of the leaves in the orchard grass seed materially decreases its commercial value, t advantage of having the clover in the field more than compensates f this.

In sections where the grass is grown for hay and pasture, red clo is sown at the rate of one bushel to 5 or 7 acres, usually as early the spring as the weather will permit. It is, however, sometimes so in the autumn at the same time as the orchard grass, but the seed the two are not mixed, as they do not feed evenly through the drill seeder. In cases where clover is sown in the spring on orchard gr that has been sown the fall before, it is either covered lightly by me of a drag harrow or left uncovered. Less clover is sown to the in seed-producing sections than where the grass is intended for hay pasture, the customary quantity being one bushel to 8 or 10 acr In these sections the first crop is frequently cut for hay on account its containing so much clover. The second crop usually contains v much less and is cut for seed, as are also the following crops, si the clover at the end of two years usually disappears. Pasturing field appears to materially increase the longevity of the clover, and is not uncommon to see meadows that are 6 or 7 years old containi almost as much clover as they did the first year.

Much trouble is now being experienced in securing a catch of clover throughout the greater part of the region where it is grow As a result alsike is being substituted in some sections, and where has been tried thoroughly it is giving good results. This difficulty growing red clover will doubtless soon become a serious propositi especially in seed-producing sections, and unless alsike or some simi leguminous crop can be grown it will be only a few years until me barnyard manure or commercial fertilizer will have to be used. the present time there is very little commercial fertilizer applied orchard grass, although it is the opinion of some of the more succe ful growers that an application of about 200 pounds of good fertili in the spring, just as the grass begins to grow, would yield profita results.

MIXTURES WITH OTHER GRASSES.

On account of the bunchy tendency of orchard grass it is oft desirable to mix it with other grasses for hay or pasture, and wh this has not been practiced as yet to any great extent the resu obtained from such mixtures are very promising. Aside from affe ing the palatability of the grass, the mixtures have a tendency increase the yield. Orchard grass matures well with tall meadow o grass and meadow fescue, and in some localities in Tennessee a mi ture of it with the latter is attracting considerable attention, especial for pasture. Doubtless in time orchard grass will be more general grown for hay and pasture in mixtures with these or other grasses.

LIFE OF MEADOWS.

Orchard grass is a more hardy and permanent grass than timothy, d as a result remains productive in a meadow under most conditions uch longer. In the principal sections where it is grown the average e of a meadow is from five to seven years, although it is a question hether it might not be broken up profitably at the end of four years. hroughout almost its entire region Kentucky bluegrass is its natural emy and works in around the bunches almost to its ultimate exclu- n. Redtop and Canada bluegrass also are present in many sections th the Kentucky bluegrass, and at the end of five or six years these ree grasses are greatly in the majority. Pasturing seems to facili- te the growth of the bluegrass, inasmuch as it has a tendency to se the orchard grass to become more bunchy, and it is also a means spreading the bluegrass seed. During the last year of its existence is customary to pasture the orchard grass field, and late in the tumn or early in the winter it is broken up and is planted to corn following spring. It is well to have the field broken up as early as ssible so as to give the sod time to rot sufficiently before planting e corn.

USES AND VALUE.

HAY.

According to chemical analysis orchard grass hay should be equal, not superior, to timothy, but in real practice it does not seem to be le to successfully compete with the latter. In large cities there is ctically no demand for any hay except timothy, and the demand for chard grass hay is only local and very limited. In the timothy gion orchard grass is looked upon very unfavorably, but where nothy can not be grown so successfully its hay is used to a greater tent and is considered of very good quality.

As previously stated, orchard grass should be sown thicker when sired for hay than for seed, 2 bushels of good seed to the acre ing usually required, for unless thick it becomes coarse and woody. s value as hay is increased by the addition of red clover or alsike, d where it has been sown with other grasses, such as tall meadow t-grass or meadow fescue, its quality seems to be improved by such xtures. The state of maturity at which the grass makes the best y is when it is just in bloom. Not only does the quality seem to be tter at this time, but the yield is also at the maximum.

In some sections it is considered a good hay for horses, but it is of ore value for cattle, and especially for fattening them for the market. s a feed for sheep it is of only fair quality. The value of the hay pends not only on the state of maturity at which it is cut, but also the bunchiness and coarseness of the grass. These characteristics are influenced largely by the method of culture, and it is often for th reason that farmers do not thoroughly understand growing it that the condemn it as a hay grass. Seeding evenly with the proper quanti of seed and careful pasturing are important factors in securing good meadow.

PASTURE.

For pasture, orchard grass gives best results in mixtures with oth grasses and clovers, and is of special importance from the fact that can be grazed early and late in the season. It is quite a valuable if for no other reason than this. It also stands grazing fairly It must, however, be closely pastured; otherwise it will beco coarse and woody, and stock will not eat it. Stock do not rel mature grass, and invariably lose flesh when turned on a field condition. To secure best results from pastures, they should be some time during June to keep down the weeds, and again needed. In this way they are kept clean and more productive. grass and white clover are usually very prominent in most grass pastures and are valuable additions, as they grow betw bunches of the orchard grass, thus increasing the yield. The clover is also of value in maintaining soil fertility.

SEED.

Orchard grass seed is produced to some extent throughout the enti region in which it is grown. There is quite a quantity raised in wes ern Virginia, but the greatest seed-producing area is in the vicinity Louisville, including Jefferson, Oldham, and Shelby counties, Ky Clark County, Ind., and some of the counties adjoining th in both States mentioned. Just why there is more seed duced in this section than elsewhere is not definitely known. are of the opinion that it is because orchard grass seeds readily there, which may be true. However, the cultural m employed by farmers in this section may have something to d the success attained in raising it. In Oldham County, Ky average production is about 55,000 bushels, which represent tically 5,000 acres, as the yield is about 10 to 12 bushels to th The growing of seed in the section referred to is a profitable in and there are many farmers who engage in it quite extensive uniform success. It is said to be a more profitable crop than and when the harvest of the two conflict orchard grass is giv most attention. The average price of seed for the last ten yea been about $1.25 a bushel. The seed alone does not represent entire return from the field, for after it is harvested the meado afford hay or pasture, or both, from which a considerable pro accrues. Orchard grass seed is the controlling crop in this sectio and the cropping system is planned to accommodate it.

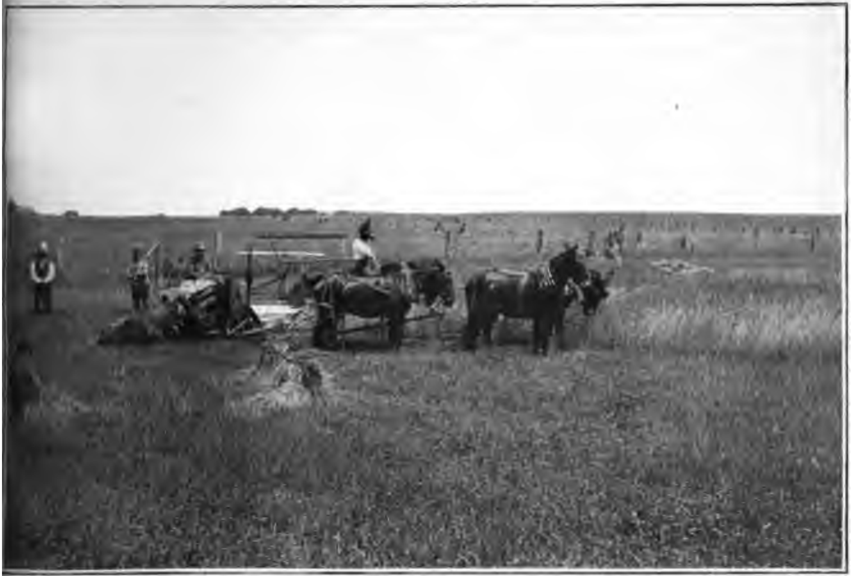

FIG. 1.—HARVESTING ORCHARD GRASS FOR SEED.

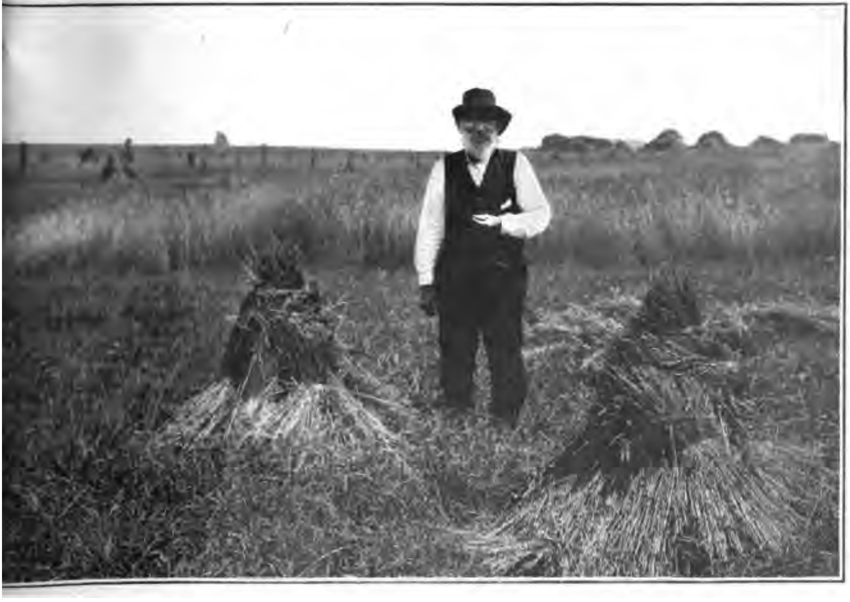

FIG. 2.—METHOD OF SHOCKING ORCHARD GRASS; SHOCKS SHOWING BANDS AT TOP.

HARVESTING THE SEED CROP.

The methods used in harvesting orchard grass seed are practically he same throughout the whole country. In general, harvest begins bout June 15 and lasts about ten days, though when there is a large creage it is often necessary to begin earlier than this, in order to finish efore the seed becomes too ripe. An inferior quality results from utting the seed before it is sufficiently mature, and this seed is quite eadily detected by its light-green color. When properly matured he seed is straw colored, and not at all green. A common test to etermine whether the seed is at the proper stage for cutting is to eat the heads in the palm of the hand, and if quite a quantity shat-ers off it is considered ready to cut. To one unfamiliar with the crop would seem that the waste from shattering would be great.

Orchard grass is harvested with an ordinary grain binder (Pl. VII, g. 1), making as small bundles as possible, in order that they may ure readily. The bundles are placed usually three in a shock and he shocks tied at the top with two bands of straw, one about 8 inches elow the other (Pl. VII, fig. 2). They are bound in this way so as to ake them more stable and to prevent the seed from shattering. The ocks are made small to facilitate handling at the time of thrashing, d so that they may be easily tied at the top with the straw bands. hey are left standing from two to four weeks, or until they have had ne to cure thoroughly, and are thrashed without stacking. On an erage it takes about 5 pounds of twine for 100 bushels of seed. The op is of such importance that the fence corners and other places that n not be reached with the binder are cut with the cradle and bound to bundles by hand.

When the grass is sufficiently tall it is cut from 12 to 14 inches high, avoid the low-growing weeds, such as plantain and sorrel, and also over and bluegrass. Another advantage in high cutting is that it aves more of the undergrowth to be utilized later for hay or pasture.

THRASHING.

The common grain separator is used for thrashing with the ordi-ry cylinder and concaves, but with special riddles and with nearly l the wind shut off to prevent too much of the seed from being blown er. In hauling the shocks to the machine, racks with tight beds or ith tarpaulins spread over the bottom are used to catch the seed that atters off, which is usually considerable. This is always heavy seed, d is worth saving. Since the shocks are small, a whole one may be rown on the rack at one forkful without breaking the bands. This duces shattering to a minimum. Unless the grass is very weedy e thrashing machine cleans the seed sufficiently for the market, but ost of the larger growers have hand fanning mills, which are used hen necessary. Seeds like those of redtop are easily blown out, but it is harder to dispose of the bluegrass and some of the weed seed such as plantain and whitetop. From the machine the seed is put int 8-bushel bags for shipping. Thrashing costs on an average 8 cen per bushel, with the customary crew furnished.

HANDLING THE AFTERGROWTH.

In cases where orchard grass is cut for seed there is a great diffe ence of opinion as to how the aftergrowth should be handled. It i generally considered that pasturing is not in the least detrimental b is even beneficial. Whether the aftergrowth should be cut for h is an undecided question. It is a common practice, however, to c it, due to the fact that it is depended upon largely for hay, since ti othy and other hay grasses are not grown to any great extent. Aft the grass is cut for seed, especially when it is cut sufficiently hig there is always considerable green undergrowth. This continues grow, and during the latter part of August or about the first of Se tember is at the proper stage to cut for hay. If there is clover pre ent in the aftergrowth it makes a very fair quality of hay and yiel from one-half to one ton to the acre. The quality of this hay is n so good as that of the hay made from the first cutting.

While it is a general practice to cut the aftermath during the latt part of August or September, there are some who prefer to cut it soon as the shocks are removed from the field, as it is believed to b better for the following seed crop if it is cut then. It is the opinio of some that the aftermath should be cut in any event, and consequentl if it is not desired for hay it is cut and left on the ground. Othe are of the opinion that if it is cut at all it materially injures the ne year's crop, especially so if used for hay, as the two cuttings remo a large amount of plant food from the soil without much return.

Judicious pasturing, to say the least, is not detrimental to the fiel and in all probability is more or less beneficial. The aftergrowt which comes on after the seed crop is removed, furnishes grazi until it is covered with snow, and in the more southern sections whe the grass is grown lasts nearly the entire winter. Sheep can be ve profitably pastured on this aftergrowth, and in many cases almost much money is made from the pasture that it affords the sheep from the seed crop, on account of the length of time which it wi furnish grazing. At present prices sheep are equally as profitable cattle, if not more so, and can be pastured on orchard grass to mu better advantage.

VALUE OF THE STRAW.

There is much difference of opinion regarding the value as a fe for stock of orchard grass straw from which the seed has been thrashe Some state that it is of almost as much value as the hay, but in gener it is thought to be about equal to wheat straw. Its value depen rgely on three factors: The state of maturity at the time of cutting; e amount of aftergrowth, including red clover, contained in it, and e success with which it is cured. If the grass is cut before the seed sufficiently mature to harvest, the straw will be of more value for ed than when it is cut at the proper stage. The undergrowth which present probably furnishes as much feed as the straw itself, if not ore, especially when it contains clover.

It is a common practice to cut the grass as high as possible to avoid e weeds and clover, but if it is short there is necessarily a great deal undergrowth cut with it. If the grass has not received too much in while in the shock and is stacked properly or put into a barn or ed at the time of thrashing, the straw will be of much more value an if carelessly handled. In general, there is little attention paid to e stacking of the straw, and it is commonly left in piles just as they e made by the machine. When utilized for forage it is fed to horses cattle, usually the latter, but is of very little value for sheep. side from its value as a feed, straw may be used for seeding meadows, previously described. It should never be used for this purpose, wever, unless thoroughly free from weeds.

WEEDS IN ORCHARD GRASS SEED FIELDS.

The weeds which are most troublesome in orchard grass fields, espe- lly in sections where seed is produced, are whitetop (*Erigeron nnuus*), red sorrel (*Rumex acetosella*), oxeye daisy (*Chrysanthemum ucanthemum*), milfoil (*Achillea millefolium*), and the plantains *Plantago lanceolata* and *P. aristata*). Most growers pay much atten- on to keeping these weeds out of their fields and go to considerable pense for labor to mow them or cut them out with a hoe just before rvest. A method which is now quite commonly used and which is st effective and practicable is to pasture the fields with sheep. This an excellent practice and it is comparatively easy to distinguish at rvest time between fields that have been pastured in this way and ose that have not by the absence of weeds in the former. Such od results have been obtained by pasturing sheep on the grass to ep down the weeds that farmers are raising more sheep than formerly d are growing cleaner seed. It is a common practice to turn the eep on in the spring as soon as the grass begins to grow and allow em to remain until the early part of May. As the grass advances ward maturity the sheep eat very little of it, but graze mostly upon e weeds and undergrowth, and especially on the whitetop, which is ne of the worst weeds present, if not the worst. They do little dam- ge to the field when it is dry, and in wet weather they are kept off, as ey drag down too much of the grass. Although it is the custom to rn the sheep out of the fields in the early part of May, some of the ost successful growers leave them in until nearly harvest time. It is not uncommon to see sheep in fields that are ready to harvest. When it is possible to do so the fields should be pastured late, as this practice is more effective in keeping down the weeds, since it takes them but a short time to make sufficient growth to interfere with the cleaning of the seed. Cattle are sometimes pastured on fields that ar intended for seed, but they tramp down too much of the grass and ar not as satisfactory for this purpose as sheep.

OTHER GRASSES IN FIELDS INTENDED FOR SEED.

Much has recently been said regarding the presence of seeds of othe grasses in orchard grass seed. Those which appear to be the mos common are meadow fescue (*Festuca pratensis*) and the rye-grasse (*Lolium perenne* and *Lolium italicum*). The seed of these grasses i much heavier, but it resembles orchard grass seed to such an exten that its presence is not readily detected. Meadow fescue and rye-gras have very much the same appearance, but there is no difficulty in distinguishing them from orchard grass, as their seed habits and genera habits of growth are different. If these grasses were present in considerable quantities in the orchard grass fields in Kentucky, Indiana, and western Virginia, where practically our entire supply of seed i produced, the presence of their seed in orchard grass seed could b readily accounted for. The orchard grass fields in these sections, however, are almost entirely free from other grasses, and only in a ver few cases are there any others present, with the exception of som Kentucky bluegrass (*Poa pratensis*) and Canada bluegrass (*Poa compressa*) and a little cheat (*Bromus secalinus*) and redtop (*Agrostis alba*) Bluegrass and redtop, especially the former, come in naturally in th older fields, and cheat is present practically only the first year, du to its having been in the wheat which just preceded the grass crop The quantity of meadow fescue and rye-grasses in these fields is insi nificant, and there are only a very few cases where these grasses a present at all. The total percentage of other grasses in orchard gras throughout the whole seed-producing section is so small as to be hardl worthy of consideration, and statements made to the effect that th presence of their seed in orchard grass seed is due to the fact that the are grown with the orchard grass and can not be separated from it a entirely without foundation. Farmers in general are extremely car ful to keep their orchard grass fields free from other grasses, for th reason that their seeds are readily detected by buyers and as a cons quence the seed invariably sells at a lower price. It is a comparativel easy matter for seed growers to have pure seed for their own sowin and there would be absolutely no advantage to them in growin meadow fescue, rye-grasses, and other grasses with orchard grass.

SUMMARY.

Orchard grass is of considerable value for early and late pasture, and in the southern part of the region where it is grown can be pastured nearly the entire year. When used for pasture, bluegrass and white clover are commonly grown with it.

Orchard grass hay is of value, especially when it contains red clover, and can be fed to horses successfully. It is a good forage for cattle that are being fattened for market.

When grown for seed, orchard grass is a profitable crop, as it yields on an average 10 to 12 bushels to the acre and sells for $1.25 a bushel. Aside from securing a crop of seed, the aftergrowth may be either pastured or cut for hay. This aftergrowth makes a very fair quality of hay, and when cut during the latter part of August or September gives a yield of from one-half to one ton to the acre.

Although not previously stated, orchard grass is quite valuable for binding soils, and on rough land that washes badly it can be used for this purpose effectively.

Orchard grass may be seeded either in the autumn or spring with about equally good results. Spring seeding, however, seems to be the most common practice. In most cases it is sown broadcast on fall wheat on fields that have been in wheat the previous year. One bushel of seed is a sufficient quantity when the grass is to be grown for seed. When grown for hay or pasture, more than this should be used. A good catch may be obtained by scattering the straw evenly and thinly on fall wheat in early spring.

Red clover can be profitably sown with orchard grass at the rate of 1 bushel to 5 or 7 acres. Mixtures of orchard grass with other grasses, especially with tall meadow oat-grass and meadow fescue, are giving good results for hay and pasture in places where they are being tried.

The average life of an orchard grass meadow is from five to seven years, after which it is plowed up, usually late in the fall, and put into corn.

Orchard grass is harvested for seed from about June 15 to June 25. It is cut with an ordinary grain binder and bound into small bundles, requiring about 5 pounds of twine to 100 bushels of seed. The bundles are put three in a shock and bound at the top with a band of grass to make them more stable and to prevent the seed from shattering. Thrashing is done from the shock after the grass has stood in the field from two to four weeks, with an ordinary separator, using special riddles.

Sheep are pastured on orchard grass in the spring to keep down the weeds. They are sometimes allowed to remain in the field until nearly time to harvest. This practice is very effective in keeping clean the fields that are grown for seed.

The percentage of meadow fescue, rye-grasses, and other grasses in orchard grass fields that are grown for seed is so small as not to be worthy of consideration.

VII.—THE EFFECT OF COPPER UPON WATER BACTERIA.[a]

By Karl F. Kellerman, *Physiologist in Charge of Soil Bacteriology and Water Purification Investigations*, and T. D. Beckwith, *Scientific Assistant.*

INTRODUCTION.

Of the many methods of chemical treatment for water purification, the copper method has recently received the most attention. The use of this metal, or its salts, was urged primarily[b] for the eradication or control of polluting algæ, though popular attention has been directed more particularly to the various results obtained in destroying the typhoid bacillus.

The discrepancies between laboratory results of some of the investigators of this phase of the subject have deterred many engineers and sanitarians from conducting important tests of the value of copper under emergency or epidemic conditions. These discrepancies are perhaps in some cases due to reasons which are taken up later in this bulletin, and in some cases to the lack of comprehension of the essential difference between treatment of drinking water which contains relatively little albuminoid matter[c] and treatment of bouillons or emulsions.[d]

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[a] The copper method for controlling algal pollution can no longer be considered in an experimental stage. The accumulation of results of treatments constantly made under the direct supervision of officials of the Department of Agriculture and the numerous results reported to this Department by independent experimenters render further discussion of this question superfluous. The caution should be reiterated, however, that no rule for determining the amount of copper sulfate to be added can be given, and each body of water must be treated in the light of its special conditions. This caution is eminently applicable to copper treatment of a water supply for bactericidal purposes, and, as Mr. Kellerman and Mr. Beckwith have shown in their recent work, the problem of using dilute solutions of copper for destroying *Bacillus coli* and *Bacillus typhi* in water is a rather complicated one. Pertinent conditions must be understood thoroughly before an application of copper can be safely advised, and whether an emergency treatment of an unfiltered and contaminated water, or a continuous treatment of a filtered water supply, or a treatment of a sewage effluent is contemplated the work should be supervised by an expert.—A. F. Woods, *Pathologist and Physiologist, Acting Chief of Bureau.*

[b] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[c] See also Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[d] A case in point is a paper by Dr. J. B. Thomas, Report on the Action of Various Substances on Pure Cultures of the Amœba Dysenteriæ. (Amer. Jour. Med. Sci., vol. 131, No. 1, Jan., 1906, p. 116.) "Uniform suspensions of the amœba were made by pouring 4 cc. of distilled sterile water over the surface of a 48-hour slant agar culture of the amœba and cholera spirillum, scraping off the surface growth and mixing with the matter by means of a platinum wire, and pouring the resultant emulsion into a sterile test tube; 4 cc. of the antiseptic solution (in double strength)

In spite of the fact that the field results of several experimenters have apparently established the point that copper sulfate will no destroy ordinary water bacteria at concentrations fatal to the colo and typhoid bacilli, the somewhat fanciful objection has been su gested to chemical treatment of any kind, and to copper treatment i particular, that bacteria desirable for oxidation of organic matter an other beneficial changes in a water supply might be injured equall with the typhoid bacteria, and thus a treated water be more pote tially dangerous than an untreated water known to contain typho organisms; in other words, that many bacteria which could be decided benefit in a slightly polluted water supply might be eradicat by copper treatment and that in this way the fractional sterilization a reservoir by copper might pave the way for a more dangerous co tamination.

Investigations of these and other points have been undertaken, a it is believed that valuable data have been obtained, applicable copper treatment for algæ, to emergency treatment for typhoid, a to copper treatment in connection with filtration. The copper tre ment of sewage may be influenced by the same conditions that be upon the effect of copper treatment of water. For the present, how ever, the investigations of Johnson,[b] at the Columbus, Ohio, sewa testing station may be considered sufficiently accurate for practic purposes.

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to be tested was then added to the 4 cc. emulsion of amœbæ, thus making a fair uniform emulsion of 8 cc. of liquid to one 48-hour slant culture, the mixture co taining a definite amount of the chemical to be tested. * * * It would appe from the above results that it would be disastrous to rely on the action of copp containers to purify water infected with amœbæ or cholera."

[a] Caird. Copper Sulphate Results. Paper read at meeting of American Wat Works Association, Boston, Mass., July 10–14, 1906.

Jackson. Journal New England Water Works Association, vol. 19, 1905, p 563–568.

Hollis. Journal New England Water Works Association, vol. 19, 1905, pp. 571–5

Stokes and Thomas. The Effect of Copper Sulphate upon the Bacteriological a Chemical Constituents of Large Bodies of Water. Public Health Papers and Repor American Public Health Association, vol. 31, part 1, 1905, pp. 75–90.

[b] The Copper Treatment of Sewage Effluents. Report on Sewage Purification Columbus, Ohio, 1905.

"Available data indicate that the removal by either process of applied pathogen and nonpathogenic bacteria is in fairly direct proportion, generally speakin although, of course, saprophytic bacteria may multiply within the tanks or filters, as to obscure the true removal. Under some conditions it might be advantageous employ a germicide, such as sulphate of copper, as a final treatment for sewage eff ents of doubtful bacterial purity." (P. 471.)

"Independent of the question of complete sterilization as touched upon * * it may be that there is a field of usefulness in some places for copper sulphate or oth germicidal chemical in the treatment of coarse-grain filter effluents, in order to bri them from a bacterial or hygienic standpoint to a degree of purity strictly compa able with that of the effluent of ordinary intermittent sand filters." (P. 479.)

RESISTANCE OF VARIOUS BACTERIA.

Several species of bacteria occurring commonly in Potomac River water have been isolated, and comparative tests have been carried on with these bacteria and with the colon bacillus. An examination of he tables showing the exact results of these tests indicates clearly hat no danger to most of the common saprophytic bacteria can be xpected from using concentrations of copper sufficiently strong to estroy *Bacillus coli.* To allow as little variation as possible in the ifferent tests all experiments were carried out in triple-distilled water. This will probably explain the sensitiveness of most of the bacteria to opper solutions, for, as Gildersleeve[a] has stated, "The resistance of acteria washed in distilled water is lessened, due in all probability to he fact that some of the slimy substance which surrounds the bacerial cell and protects it to some extent from the action of detrimental gencies is removed." For purposes of comparison the table showing he action of copper sulfate on various bacteria in tap water, pubished by Gildersleeve,[b] is reproduced below.

Organism.	Dilution.	Average number to the cubic centimeter in control.	Percentage destroyed in hours specified.												
			1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	24.
B. typhosus	1: 250,000	110,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	100,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	140,000	78	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	120,000	50	85	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	130,000	4	7	55	80	82	87	87	93	98	100	....	....	....
B. coli communis	1: 250,000	105,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	99,000	80	99	100	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	150,000	87	95	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	110,000	8	10	14	27	50	85	90	92	93	93	95	98	100
B. dysenteriæ	1: 250,000	11,500	95	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	40	95	97	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	72,000	31	50	62	85	99	99+	100	....	....	....	....	....	....
	1:1,500,000	12,400	20	30	40	42	60	70	75	81	85	87	90	91	100
	1:2,000,000	13,600	20	20	25	30	30	45	60	62	62	68	71-	75	98
B. cloacæ	1: 250,000	92,000	90	99	100	....	....	....	....	....	....	....	....	....	....
	1: 500,000	86,000	68	90	99	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	101,000	33	85	89	96	96	99	100	....	....	....	....	....	....
	1:1,500,000	89,000	5	10	30	40	70	85	95	98	98	99	100	....	....
	1:2,000,000	94,500	3	10	31	40	80	83	85	88	91	91	93	97	100
B. proteus vulgaris	1: 250,000	115,000	93	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	106,000	65	92	98	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	111,000	28	51	82	90	95	98	100	....	....	....	....	....	....
	1:1,500,000	99,000	10	18	27	42	69	90	93	98	99+	100	....	....	....
	1:2,000,000	118,000	0	12	22	36	51	65	78	80	85	88	92	95	100
B. prodigiosus	1: 250,000	102,000	95	99	99	99+	100	....	....	....	....	....	....	....	....
	1: 500,000	109,900	15	26	80	95	97	99	99+	100	....	....	....	....	....
	1:1,000,000	110,000	9	11	16	33	33	45	47	61	63	69	....	....	93
	1:1,500,000	107,000	4	12	17	30	38	46	46	48	51	52	60	61	92
	1:2,000,000	98,000	0	1	8	8	10	11	15	30	38	41	43	50	88
Staphylococcus pyogenes aureus	1: 250,000	92,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	88,000	50	70	90	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	86,000	15	30	30	70	78	85	97	99	99	100	....	....	....
	1:1,500,000	100,000	8	22	28	96	58	69	85	93	95	99	100	....	....
	1:2,000,000	95,000	1	8	10	30	50	64	79	82	85	98	99	99+	100

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[a] Studies on the Bactericidal Action of Copper on Organisms in Water. American ournal of Medical Science, vol. 129, 1905, p. 754.

[b] Op. cit., p. 759.

The figures in this table are rather surprising in connection wit the statement that "very little difference was found between distille and filtered water used in the laboratory." The similarity here of th action of copper in distilled and filtered tap water, contrasted with th marked difference in the action of distilled and filtered tap water i Washington, emphasizes a point previously mentioned,[a] that the wate itself deserves as careful a study as do the organisms contained therei This is suggested again in Phelps's[b] report showing that with eith hard or turbid waters the germicidal efficiency of metallic copper i much lessened. Again, in view of the high toxicity that investigato have reported with copper in Philadelphia tap water, it seems neces sary to assume that this water either is peculiarly favorable to mai taining the metal in a toxic state, or, what seems equally probabl for rendering the bacteria unusually sensitive.

The results of our own experiments are given in the following table All the experiments, unless otherwise indicated, were conducted i Weber resistance glass test tubes, each containing 10 c. c. of wat triple distilled from glass, portions of which had been treated pre viously with the desired amount of copper sulfate. All tubes wer inoculated with a 2 mm. loop of the proper organism. The temperature during each experiment varied from 18° to 22° C.

TABLE I.—*Effect of copper sulfate upon Bacillus mycoides.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,00 parts o water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	105	90	590	310	70	250	[illegible]
6 hours	80	1	20	80	15	35	[illegible]
24 hours	185	10	130	110	40	20	[illegible]

TABLE II.—*Effect of copper sulfate upon Bacillus megatherium.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,0 parts water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	70	55	45	30	90	15	[illegible]
2 hours	50	10	25	30	40	0	[illegible]
6 hours	40	15	20	10	40	1	[illegible]
24 hours	200	10	15	5	10	2	[illegible]

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[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture, p. 12; and Kelle man, Journal New England Water Works Association, vol. 19, 1905, p. 536.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 537–539.

TABLE III.—*Effect of copper sulfate upon Bacillus mesentericus.*

ration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
hour	300	380	400	520	280	440	470
hours	280	170	310	440	260	340	360
ours	430	10	290	320	250	310	390
hours	1,350	0	0	530	3	4	30

TABLE IV.—*Effect of copper sulfate upon Bacillus mesentericus fuscus.*

ration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	30	40	25	25	50	20	30
ours	0	5	50	10	3	0	1
ours	2	1	30	5	5	2	1
hours	70	110	70	45	0	10	80

TABLE V.—*Effect of copper sulfate upon Streptococcus pyogenes.*

ration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	180	875	875	300	250	90	180
ours	210	0	0	290	180	180	180
ours	140	0	0	280	230	240	160
hours	35	0	0	390	150	10	20

TABLE VI.—*Effect of copper sulfate upon Bacillus subtilis.*

ration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	310	80	20	130	85	100	30
ours	220	1	3	30	120	70	10
ours	330	1	1	25	85	50	2
hours	2,800	1	0	30	70	65	0

TABLE VII.—*Effect of copper sulfate upon Bacillus prodigiosus.*

ration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	350	450	400	1,050	22,000	2,000	1,800
ours	750	65	180	1,700	1,350	2,050	1,550
ours	1,250	4	0	2	0	550	200
hours	925	0	0	0	0	0	0

TABLE VIII.—*Effect of copper sulfate upon Bacillus liquifaciens phosphorescens.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,00 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies*
0 hour	16,500	3,400	2,400	16,000	4,500	10,400	13,
2 hours	15,800	0	1	0	5	2,900	7,
6 hours	23,000	0	0	0	0	640	7,
24 hours	36,000	0	0	0	0	60	5,

TABLE IX.—*Effect of copper sulfate upon pink yeast.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,0 parts of wate
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies*
0 hour	310	550	100	110	100	220	[illegible]
2 hours	150	5	1	0	1	65	[illegible]
6 hours	70	0	0	0	1	3	[illegible]
24 hours	90	0	0	0	0	1	[illegible]

TABLE X.—*Effect of copper sulfate upon Bacillus coli.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.	2,000,000 parts of water.	3,000,000 parts of water.	4,000,0 parts o water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colmies.*	*Colonie*
0 hour	2,650	2,300	3,100	2,700	2,250	1,800	1,
2 hours	2,350	1,650	2,650	2,000	1,650	1,850	1,
6 hours	2,300	0	0	95	210	850	1,
24 hours	6,300	0	0	0	0	0	[illegible]

TABLE XI.—*Effect of copper sulfate upon sulfur yellow bacillus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,0 parts water
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Coloni*
0 hour	2.800	40	3,100	2,800	4,100	860	1,
2 hours	2,000	0	0	0	280	1	[illegible]
6 hours	1,500	0	0	0	0	0	[illegible]
24 hours	24,000	0	0	0	0	0	[illegible]

TABLE XII.—*Effect of copper sulfate upon Pseudomonas radicicola (soy).*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,0 parts water
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Coloni*
0 hour	1,900	520	1,300	2,800	650	520	3,
2 hours	1,800	0	0	45	0	25	1,
6 hours	2,100	0	0	0	0	0	[illegible]
24 hours	18,000	0	0	0	0	0	[illegible]

TABLE XIII.—*Effect of copper sulfate upon Bacillus sublanatus.*

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	12,500	780	2,700	2,100	1,100	2,100	1,500
ours	12,000	0	0	0	0	35	190
ours	12,500	0	0	0	0	0	80
ours	40,000	0	0	0	1	0	0

TABLE XIV.—*Effect of copper sulfate upon Micrococcus radians.*

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	1,200	2,700	10,500	1,700	3,800	2,500	2,800
ours	1,200	1	0	1	20	85	10
ours	1,400	0	0	0	0	0	1
ours	1,900	0	0	0	0	0	0

TABLE XV.—*Effect of copper sulfate upon Pseudomonas radicicola (alfalfa).*

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	270	300	330	425	3,700	700	290
ours	210	0	0	0	0	0	0
ours	220	0	0	0	0	0	0
ours	1,175	0	0	0	0	0	0

TABLE XVI.—*Effect of copper sulfate upon Bacillus violaceus laurentius.*

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	7,200	120	480	740	470	210	520
ours	5,300	0	0	0	0	1	25
ours	6,800	0	0	0	0	0	0
ours	40,000	0	0	0	0	0	0

TABLE XVII.—*Effect of copper sulfate upon Pseudomonas amethystina.*

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
ur	140	540	90	70	580	670	660
urs	190	0	0	1	3	3	15
urs	240	0	0	0	0	1	1
ours	....	0	0	0	0	0	0

TABLE XVIII.—*Effect of copper sulfate upon Bacillus caudatus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,00 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	875	60	680	2,100	15	
2 hours	350	0	0	1	0	
6 hours	400	0	0	1	0	
24 hours	9,000	0	0	0	0	

TABLE XIX.—*Effect of copper sulfate upon Bacillus rubrum.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,00 parts o water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	175	10	30	225	90	210	1
2 hours	195	0	0	90	0	0	
6 hours	50	0	0	5	0	0	
24 hours	1,050	0	0	5	0	0	

EFFECT OF CARBON DIOXID ON VIABILITY OF BACILLUS COL AND BACILLUS TYPHI.

A careful study of the gas content of both tap and triple distille water has shown that for the typhoid and colon bacilli the presence o carbon dioxid in the water is associated with heightened resistance t toxic agents, such as solutions of copper salts, precipitated coppe salts, and copper metal. This is the more strange when one consider that the presence of carbon dioxid in water causes the copper t remain in solution, and in case of insoluble copper, either precipitate or metallic, the carbon dioxid serves to bring a considerable amoun of copper into solution. It should be noted, however, that wate heavily saturated with carbon dioxid is toxic to *Bacillus coli* an *Bacillus typhi.*

A series of experiments designed to test the effect of carbon dio on *Bacillus coli*, the various conditions of triple distilled water wi and without copper, triple-distilled water plus calcium carbonate wi and without copper, and tap water with and without copper are tabulated below.

TABLE XX.—*Toxicity of copper sulfate to Bacillus coli in the absence of carbon diox*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,00 parts o water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	6,800	1,400	2,300	2,200	900	3,5
2 hours	5,100	0	0	0	2	

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[1] Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water tri distilled from glass, portions of which had been treated previously with the desired amount copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received fr Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

ABLE XXI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

ation of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
ur	9,200	8,200	5,800	11,600	7,500	6,200
urs	2,000	0	110	38	900	350

Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple lled from glass, portions of which had been treated previously with the desired amount of copper te. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theo-Smith. The temperature during this experiment varied from 18° to 22° C.

BLE XXII.—*Effect of water containing calcium monocarbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

tion of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
ur	2,950	3,510	2,670	3,150	4,290	3,760
rs	240	0	0	0	105	80
urs	5	0	0	0	0	0

arious dilutions of copper sulfate were tubed in Weber resistance glass, thoroughly boiled, a small ured quantity of calcium carbonate added to each tube, and these solutions cooled and kept in vy jar in an atmosphere free of carbon dioxid. All tubes inoculated with a 2 mm. loop of cul-of *Bacillus coli* received from the Bureau of Animal Industry and isolated from hog. The tem-ture during this experiment varied from 18° to 22° C.

LE XXIII.—*Effect of carbon dioxid content of water containing calcium carbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

tion of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
r	4,650	4,730	3,850	3,980	4,100	4,470
rs	4,500	0	0	5	415	620
urs	5,200	0	0	0	15	60

rious dilutions of copper sulfate were tubed in Weber resistance glass, thoroughly boiled, a measured quantity of calcium carbonate added to each tube, and these solutions cooled and n a Novy jar in an atmosphere composed largely of carbon dioxid. All tubes inoculated with m. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry and isolated hog. The temperature during this experiment varied from 18° to 22° C.

LE XXIV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxid.*[1]

tion of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
r	6,300	7,600	6,000	1,050	4,800	5,200
rs	1,500	0	0	0	Lost.	60
rs	280	0	0	0	0	0
urs	0	0	0	0	0	0

periment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap , portions of which had been treated previously with the desired amount of copper sulfate. bes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal stry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxi*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000, parts wate
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colon*
0 hour	4,600	1,800	4,800	3,700	4,500	4,
2 hours	1,450	0	0	20	80	
6 hours	1,500	0	0	0	5	
24 hours	250	0	0	0	0	

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac water, portions of which had been treated previously with the desired amount of copper sul All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald S The temperature during this experiment varied from 18° to 22° C.

TABLE XXVI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus co*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000, parts wate
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colon*
0 hour	4,200	4,000	9,800	1,270	5,100	1,
2 hours	2,800	10	0	0	110	
6 hours	1,460	15	15	20	10	
24 hours	1,540	0	0	0	0	

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac water, portions of which had been treated previously with the desired amount of copper sul All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of An Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXVII.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus co*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000, part wat
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colon*
0 hour	3,800	3,000	8,500	5,200	4,400	5
2 hours	2,300	0	5	15	2,150	1
6 hours	1,600	10	12	10	120	
24 hours	3,500	0	0	10	35	

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potoma water, portions of which had been treated previously with the desired amount of copper su All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald S The temperature during this experiment varied from 18° to 22° C.

An examination of the foregoing tables shows that with the th types of water the presence of carbon dioxid increases the resistance the bacilli in question. In the solution containing monocarbonate lime and copper sulfate the bacteria are extremely sensitive, even bacteria in the check solutions dying rapidly, while in the soluti charged with carbon dioxid the bacteria were able to persist in c siderable numbers in the dilute copper solutions in spite of the f that most of the copper must have remained in solution. This p is interesting in connection with the work of Engels,[a] who report ather high toxicity for calcium chlorid, and of Pfuhl,[a] who suggests calcium salt—milk of lime—for water sterilization. The use of lime r a similar agent may be highly desirable in connection with treating contaminated reservoir with copper, though the inferior germicidal ower of lime makes it improbable that the latter alone could be used fely.

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[a] Weitere Studien über die Sterilization von Trinkwasser auf chemischen W Centralbl. f. Bakt., Parasit., u. Infekt., vol. 32 Orig., 1902, pp. 495–521.

In regard to chemical water analysis, it seems probable that the termination of carbon dioxid or the determination of monocarbon- e and bicarbonate alkalinity may have importance hitherto unrecog- zed, and the variations in the longevity determinations of *Bacillus coli* d *Bacillus typhi* may be due in part to the mineral constituents of a ater, in part to methods of experimentation, and in part to the car- on dioxid content. Extended field tests must be made before gen- alizations on the possible effect of the gas content of a water supply n be determined.

The carbon dioxid content of a water may possibly explain the culiar results obtained by Clark and Gage.[b] They have reported actically no toxic action from metallic copper, or at least very little ifference in the action of metallic copper, iron, tin, zinc, and lead. heir figures are rather misleading because of the great number of ys the experiments were carried on, and, as Phelps[c] has shown, etallic copper is coated with some insoluble substance after a few days' posure to Boston tap water and no longer has great toxic action. e lack of toxicity of metallic copper and the similarity of its action the action of other metals, as reported by Clark and Gage, is tirely at variance with the work of Kraemer,[d] Pennington,[e] Gil- rsleeve,[f] Stewart,[g] and Moore and Kellerman.[h] Investigators nerally have agreed that it would be possible to practically sterilize drinking water by exposing it to the action of clean metallic copper. Perhaps this opinion should be qualified by adding that the action i more rapid if the water contains no free carbon dioxid, although th following tables show only slight differences between the toxicity o metals because of the presence or absence of carbon dioxid.

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[a] Über die Disinfection der Typhus and Cholera-ausleerungen mit Kalk. Zeitschr. Hyg. u. Infekt., vol. 6-8, 1889, pp. 97-104.

[b] The Use of Copper Sulphate in Water Filtration. Journal of Infectious Diseases. pplement No. 2, February, 1906, pp. 172–174.

[c] Experiments on the Storage of Typhoid Infected Water in Copper Canteens. blic Health Papers and Reports, American Public Health Association, vol. 31, part 1905, pp. 75–90.

[d] Copper Treatment of Water. American Journal of Pharmacy, vol. 76, December, 4, pp. 574–579.

The Use of Copper in Destroying Typhoid Organisms and the Effects of Copper on n. American Journal of Pharmacy, vol. 77, June, 1905, pp. 265–281.

[e] The Action of Electrically Charged Copper Upon Certain Organisms in Water. erican Journal of Medical Science, vol. 129, 1905, pp. 751–754.

[f] Studies on the Bactericidal Action of Copper on Organisms in Water. American rnal of Medical Science, vol. 129, 1905, pp. 754–760.

[g] A Study of the Action of Colloidal Solutions of Copper upon Bacillus Typhosus, erican Journal of Medical Science, vol. 129, 1905, pp. 760–769.

[h] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

TABLE XXVIII.—*Effect of carbon dioxid upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Coloni*
0 hour	3,700	1,100	3,300	3,050	3,700	3,
4 hours	440	75	30	125	275	
8 hours	305	3	3	30	120	

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water tri distilled from glass, to portions of which were added sterile blocks of the proper metals, each havi approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacil coli* received from Prof. Theobald Smith. The temperature during this experiment varied from to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

TABLE XXIX.—*Toxicity of metals to Bacillus coli in the absence of carbon dioxid.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	2,950	2,350	1,200	1,450	1,300	1,6
4 hours	965	10	10	65	40	7
8 hours	740	0	0	5	5	

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water tri distilled from glass, to portions of which were added sterile blocks of the proper metals, each havi approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacil coli* received from Prof. Theobald Smith. The temperature during this experiment varied from to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

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[a] There is a seeming discrepancy in this statement due to the fact that Mr. Ea B. Phelps, assistant hydrographer, U. S. Geological Survey, has carried on expe ments on the storage of typhoid infected water in copper canteens, some results which, with conclusions of a nature unfavorable to the use of metallic copper practically sterilizing water, were issued in a press circular of the Geological Surve A quotation from Mr. Phelps's paper paralleled with a quotation from Bureau Plant Industry Bulletin No. 76 shows clearly that Mr. Phelps's work is a corrobo tion instead of a contradiction of the fact reported in Bulletin No. 76 that meta copper has a high germicidal value. Mr. Phelps has stated: "The fact that organis do survive the copper treatment even in small numbers [Per cent reduction usual over 99.999.—K. F. K.] seems, in the writer's view, to lessen considerably the val of the canteen as a safeguard against typhoid infection. A second point of inter is the fact that the efficiency of the canteen decreases as time goes on, probab owing to the accumulation on the surface of the copper of a film of basic carbon or other insoluble copper compound." A paragraph from Bureau of Plant Indus Bulletin No. 76 reads as follows: "Complete sterilization is a standard to which ev the best filters seldom attain, and under the most unfavorable conditions the redu tion in the number of bacteria in water exposed to the action of metallic copper i twelve hours will be approximately as great as filtered water. The copper must kept clean, not, as is popularly supposed, to protect the consumer from cop poisoning, but because it is possible for the metal to become so coated with fore substances that there is no longer any contact of copper and water, and hence antiseptic action."

To determine whether the peculiar variation in the germicidal power f solutions or metals is due to the use of ordinary laboratory glassware ade of a rather soluble glass instead of carefully selected highly soluble glass, a parallel series in good and poor glass was carried on. he following tables show the slight difference in results:

TABLE XXX.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

ration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	2,600	1,925	1,850	2,450	2,600	3,100
ours	2,650	775	2,100	715	2,800	2,300
ours	2,700	525	2,350	70	2,450	4,200
hours	28,000	15	4,350	1	13,750	12,000

Experiment conducted in ordinary glass test tubes each containing 10 c. c. of water triple distilled n glass, to portions of which were added sterile blocks of the proper metals, each having approxitely 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* eived from Prof. Theobald Smith. The temperature during this experiment varied from 18° to ° C.

TABLE XXXI.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

ration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
our	2,300	4,825	4,750	2,400	2,800	3,000
ours	1,572	975	2,400	200	1,800	3,600
ours	1,400	190	3,050	20	2,000	3,850
hours	10,825	1	6,700	1	19,000	14,000

Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water triple tilled from glass, to portions of which were added sterile blocks of the proper metals, each having proximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° 22° C.

COPPER SULFATE AND FILTRATION.

The use of copper sulfate in connection with filtration has been entioned in previous bulletins. Further experiments in this field ow that in mechanical filtration with alum it is necessary to limit e use of copper sulfate to treatment some hours before coagulaon. When solutions of aluminum sulfate and copper sulfate are ixed and alkali or hard water is added in quantities sufficient to ause precipitation the copper is coagulated at once, while the aluinum is deposited on the copper and incloses it, with the result that e copper-alum coagulum is no more toxic than is the pure alum coaglum. When copper and iron salts are precipitated together the everse of this seems to take place and the precipitate retains its toxic roperties.[a]

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[a] See also H. W. Clark, Sulphate of Alumina as a Germicide. Thirty-sixth Annual eport of State Board of Health of Massachusetts, 1904, p. 288.

The following table shows the various combinations of precipitate tested and the exact results:

TABLE XXXII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Copper and alu minum hydrat
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Coloni*
0 hour	2,250	1,525	645	7,750	3,450	1,7
2 hours	675	0	230	1,800	25	3
6 hours	465	0	230	195	0	
24 hours	165	0	230	5	0	

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theob Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXXIII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Coppe and al minu hydrat
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonie*
0 hour	3,350	380	1,065	2,650	3,100	2,3
2 hours	305	0	1,900	3	1	8
6 hours	350	1	1,350	1	0	
24 hours	200	0	850	1	0	

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau o Animal Industry, isolated from hog. The temperature during this experiment varied from 18° t 22° C.

The presence or absence of carbon dioxid is probably important i this connection. If the laboratory results will hold for field conditions, a copper precipitate or a precipitate of iron and copper wil be highly toxic to *Bacillus coli* and *Bacillus typhi* in a water whos alkalinity is chiefly monocarbonate; and, conversely, the action of a copper precipitate or a copper-iron precipitate will be reduced if a wate contains free carbon dioxid. This is probably the reason that smal quantities of copper are toxic[a] in a mechanical filter using the prope quantities of iron and copper, and gives an additional reason for th advice given by Ellms[b] and Brown[c] that before filtration or distribution of a copper-treated water all free carbon dioxid and part of th semicombined carbon dioxid should be neutralized by caustic lime Copper treatment of water previous to slow sand filtration should b made under similar conditions, and as this is seldom practicable it i perhaps advisable to limit the use of copper in connection with slo sand filtration to treatment after passing the filter, and before distribution the proper quantity of caustic lime may be added. An excel nt example of the results to be expected from incorrect use of copper connection with slow sand filtration is furnished in Clark's[a] report. ot only was copper found in the effluent of the experimental filter, ut *Bacillus coli* was found in a rather high per cent of the bacterial mples.

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[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 496–503.

[c] Journal New England Water Works Association, vol. 19, 1905, p. 578.

The carbon dioxid content of the Ohio River is a possible explana-on also of the fact reported by Brown[b] that samples of Ohio River ter yielded between 0.00772 and 0.00657 grain of metallic copper to the llon, though *Bacillus coli* was occasionally present. To use his own ords, "It is seen from the work done, as above stated, that the copper present in an insoluble form and probably as the oxid united with e suspended mineral matter which the water carries. If in this form would be unable to exert any germicidal power, and, as far as could e determined, this seems to be the case. It was decided that the cop-er was present in a spent condition." As there was no monocarbon-e alkalinity noted, the Ohio River water at this time doubtless ntained some free carbon dioxid, and from the analogy of the labora-ory results it seems fair to assume that Brown's explanation must e supplemented by the theory of the heightened resistance of *Bacil-s coli* due to carbon dioxid.

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[a] Journal New England Water Works Association, vol. 19, 1905, pp. 503–505.

[b] The Purification of Water at Marietta, Ohio. Official Report to Board of Public rvice, 1906.

B. P. I.—233.

VIII.—CONDITIONS AFFECTING LEGUME INOCULATION.[a]

By Karl F. Kellerman, *Physiologist in Charge of Soil Bacteriology and Water Purification Investigations*, and T. R. Robinson, *Assistant Physiologist.*

INTRODUCTION.

The widespread use of bacteria for inoculating leguminous crops has made possible more accurate study of the conditions under which a particular species of legume might be successfully inoculated and the conditions under which failure to obtain inoculation might be expected. Inoculation tests carried on in different soils and under different cultural and climatic conditions but using the same stock culture have shown results so conflicting in certain cases that one is bound to be misled by any general statement based on one factor alone, such, for instance, as the condition of the bacteria used.

To accept as indicative of the general usefulness of pure cultures results obtained on a single type of soil is clearly opposed tô progress along a line of investigation now recognized as closely connected with soil fertility problems. The advantage of numerous tests covering many types of soil becomes increasingly apparent. The effect of prevailing cultural practices is no less to be considered; aeration by cultivation, the use of lime, and the effect of such factors on the bacterial flora, the bacteriological testing of the soil itself—all these problems call for extensive field experiments to determine how far they may become part of practical routine for successful farming.

The work here recorded emphasizes the intimate connection of soil bacteriology with certain phases of soil fertility. Soil fertility is a strictly relative term; it is possible for a soil to be fertile in regard to one crop and unfertile in regard to another, the conditions of temperature and moisture being optimum in each case. The interaction between prevailing soil conditions and biological phenomena becomes apparent at once and lends confirmation to the belief that in practice œcological conditions may be so modified as to promote the activity of the desirable organisms and retard the development of the undesirable, although under certain conditions the control of the bacterial flora undoubtedly will depend upon the introduction of virile cultures of desirable bacteria.[a]

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[a] The identification of soil bacteria and their distribution in and correlation with different types of soil, the changes in the bacterial flora with different modes of treatment and different systems of rotation, the individual and combined action of the species in producing changes in the soil favorable or unfavorable to plant growth, the introduction of favorable species and groups of species and their improvement by selection or special cultivation, and the elimination of unfavorable forms are problems calling for extended investigation; but with the information already at hand and work under way both in this country and in Europe it is not too much to expect large increases in our present knowledge along these lines.—B. T. Galloway, *Pathologist and Physiologist, and Chief of Bureau.*

USE OF LIME.

Samples of soil have been obtained from fields in various parts of the United States where attempts to inoculate legumes have failed, and greenhouse tests have been made combining various quantities of air-slaked lime with these soils. The effect of applying lime to certain types of soils is rather striking when nodule formation is considered.[b] This is especially true of those soils which give an acid reaction to litmus,[c] although several of these unfavorable soils did not give an acid reaction to litmus, but were nevertheless benefited by lime. Outlines of a few typical cases selected from our experiments follow.

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[a] The fact as shown in numerous cases that legumes can be successfully inoculated by the use of pure cultures of the nodule-forming organisms with a consequent large gain in yield and amount of nitrogen fixed is in conflict with the statement that "An enhancement of the desirable bacterial activities of the soil can only be encouraged by the proper improvement in the physical and chemical composition of the soil." (Lipman, "The Measure of Soil Fertility from the Nitrogen Standpoint," N. J. Agr. Expt. Sta. Rept., 1905, p. 243.)

[b] Fruwirth. Neue Impfversuche mit Lupinen. Deutsche Landw. Presse, vol. 18, 1892, pp. 18 and 127.

Fruwirth. Dreijährigen Impfversuche mit Lupinen. Deutsche Landw. Presse, vol. 19, 1893, p. 6.

Heinrich. Action de la chaux sur les lupins. Zweit. Ber. Landw. Versuchsst, 1894, p. 272.

Passerini. Sur l'influence améliorante des légumineuses dans des sols de compositions différentes. Staz. Sper. Agr. Ital, vol. 30, 1897, p. 68.

Deherain and Demoussy. Recherches sur la végétation des lupins; deuxieme partie, Lupins bleus. Ann. Agron., vol. 26, 1900, p. 169.

Deherain and Demoussy. Études sur les légumineuses de grande culture. Lupins jaunes. Ann. Agron., vol. 28, 1902, p. 449.

Bilwiller. Cited by Miller. Journal Roy. Agric. Soc. England, 1896, pp. 236 and 423.

Mazé. Les microbes des nodosités des légumineuses. Ann. Inst. Pasteur, vol. 13, 1899, p. 145.

Salfeld. Vernichtung der Leguminosenpilze durch Ætzkalk. Deutsche Landw. Presse, vol. 21, 1894, p. 785.

Tacke. Action de la chaux vive sur les bactéries des tubercules des légumineuses. Centralbl. f. Agriculturchemie, vol. 25, 1896, p. 297.

[c] G. A. Billings (N. J. Agr. Expt. Sta. Rept., 1905, p. 358) shows the efficient action of lime in promoting the growth of alfalfa and that the vigor of the plants seemed to be correlated with the form and greater abundance of root nodules. He suggests that soil acidity, conditions of ventilation or soil porosity, and different bacteria which may influence the form of the nodules are determining factors, controlled at least partially by the action of lime.

A quantity of soil was obtained from Blue Hill, Me., where the culture tried last year had shown no benefit with garden peas. One portion of this soil was placed in ordinary greenhouse pots; a second portion was thoroughly mixed with pulverized lime at the rate of 1 ton to the acre, approximately, and placed in similar pots, and inoculated seed sown in half of each series. Of 15 plants unlimed, 10 were without nodules, 5 having a single nodule each; of 17 plants limed, 15 were well noduled and 2 apparently free, but nodules were evident on close examination. The uninoculated pots in the unlimed series had no nodules; in the limed series there were only a few scattering nodules, the majority of the plants, 10 out of 18, being free.

This experiment was repeated, the soil itself being inoculated with the liquid culture, with results even more striking. Of 17 plants unlimed, 12 were without nodules, 5 having a total of 8 nodules; of 16 plants limed, all were well noduled, having a total of 87 nodules. The uninoculated pots in the unlimed series had no nodules; in the limed series, 15 out of 17 plants had no nodules, the other two having a single nodule each.

The combination of liming and inoculation was again strikingly shown with alfalfa grown in a poor sandy soil from Lanham, Md. In this case it seems reasonable to assume that alfalfa bacteria of low virility were present in the soil. Under the normal unfavorable soil conditions the native bacteria were unable to produce inoculation, and the virile ones from the pure culture, while able to inoculate the alfalfa plants, could benefit them only slightly. In the limed soil, however, the native bacteria were able to produce nodules in considerable numbers and to be of moderate benefit to the plants. The pure cultures of virile bacteria under similar conditions, however, caused more than double the increase that may be ascribed to the native bacteria.

The following table shows the relative virility of the two types of alfalfa bacteria in soil from Lanham:

Treatment.	Number of plants.	Number of nodules.	Average height.	Average dry weight.
			Inches.	*Grams.*
Lime:				
Inoculated	9	12	15.5	0.39
Uninoculated	13	15	13	.15
No lime:				
Inoculated	10	11	10	.09
Uninoculated	9	0	9	.06

Similar results were obtained with a large number of soils concerning which unfavorable reports had been received regarding attempts at inoculation with cultures furnished by the Department of Agriculture. Field observations on the nodule formation of various legumes showed the effectiveness of using lime or some other agent to counteract the influence of substances in the soil unfavorable to the growth of the bacteria.[a]

EFFECT OF SOIL CONDITIONS UPON BACTERIA.

The constitution or character of the soil solution has great effect upon the growth of nodule bacteria as well as upon the formation of nodules. In the large number of soil samples tested simultaneously in the greenhouse and in the laboratory it seems to hold true generally that the possibility or impossibility of securing effective inoculation on a particular crop in a certain soil can be predicted by the relative growth of the specific culture in the sterile extract of the soil in question. There also seems to be a somewhat close relation between the soil solution necessary for the growth of host and bacteria, so that in many cases, at least, it is possible to determine the suitability of a legume crop to a certain soil by the growth of the specific culture of *Pseudomonas radicicola* in the extract of the soil. The probability of an extract not truly representing the soil solution and the disturbing factor of sterilizing certain extracts will in some cases introduce error in interpreting results. The use of sterilized soil extracts as culture media for the bacteria has been found to give fairly true indications of the growth of the respective hosts of these bacteria in a considerable series already tested, however, and contemplated improvements in technique will doubtless increase the probability of securing trustworthy indications. The testing of the soil extract in its behavior toward specific bacteria may even indicate that some treatment is necessary, but can not prescribe the method of amelioration, and for the present direct experimentation is necessary to determine the proper treatment of an unsuitable soil.

As an example of the relation between the growth of the specific bacteria in the sterile soil extract and the growth of the legume host in this soil may be cited the following result of a fairly representative test, using different quantities of lime on an unfavorable soil which responded favorably to treatment with lime:

Quantity of lime.	Green weight of plant.	Number of nodules.	Number of bacteria in a cubic centimeter of soil extract.
	Grams.		
Unlimed	2.5	31	87
Limed:			
2,000 pounds to the acre	5.5	68	71,950
4,000 pounds to the acre	8.5	88	128,650

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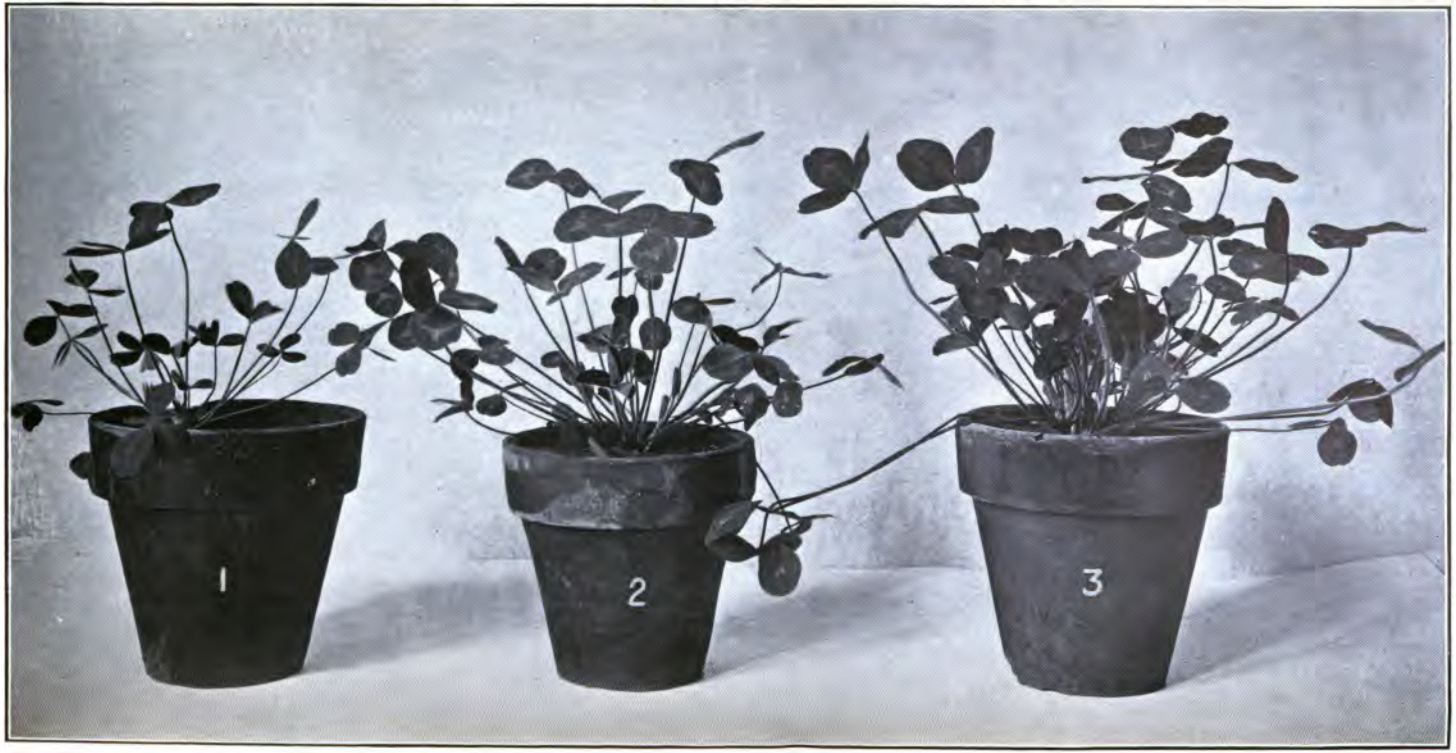
[a] For the presence of substances in poor soils deleterious to higher plants, see Bureau of Soils Bul. No. 28, 1905, "Studies on the Properties of an Unproductive Soil."



POTS OF RED CLOVER, SHOWING THE EFFECT OF LIME ON A SOIL UNFAVORABLE TO THE GROWTH OF CLOVER.

1.—Unlimed. 2.—Limed at the rate of 2,000 pounds to the acre. 3.—Limed at the rate of 4,000 pounds to the acre.

The accompanying illustration (Pl. VIII) shows characteristic pots of red clover from a series of soils from South Salem, N. Y., where clover had been steadily diminishing in vigor for years. The nodules on the unlimed plants were small and clustered around the crown of the plant, while the limed plants showed numerous nodules well distributed. The stand secured in a series of limed pots was four times as heavy as without the use of lime.

In comparison with this unfavorable soil, the extract obtained from soil sent in from a favorable field test at Carlisle, Pa., gave an excellent growth of the red clover bacteria, and the inoculated red clover grown in this soil in pots showed numerous nodules and a better growth than in the uninoculated pots, thus confirming results obtained in the field.

It may be noted that there is a difference exhibited among different legumes in their behavior toward lime. While for most legumes its effect is beneficial, a few species, such as lupine and serradella, are actually injured by its use[a] and some other legumes, such as Lima bean and cowpea, do not respond to lime even in soils where clover refuses to grow without lime. It has been noted[b] that serradella is not successful when following red clover, and that results are also unsatisfactory when red clover follows serradella. These varying relations shown by legumes belonging to different groups in their relation to lime and other conditions emphasize the necessity of determining as accurately as possible the adaptability of different soils to the crops for which they are intended.

Observations upon the occurrence of nodules in certain soils have been found to correspond quite closely with the behavior of the soil solution toward the bacteria of the legumes tested. The preceding table illustrates this point, though perhaps not so clearly as the following examples.

In the case of a fairly rich garden soil, soy beans would not form nodules even after very heavy inoculation, and the extract from this soil used as a culture medium was found to inhibit the growth of the soy bean bacteria. On the other hand, alfalfa readily formed nodules in this soil and the soil extract was not unfavorable to the growth of the alfalfa bacteria.

A soil from Niles, Mich., which had grown a fair crop of soy beans for four years without the occurrence of nodules, was tested. The soil extract in this case was found unfavorable to the growth of the soy bacteria. In greenhouse pot tests soy beans of several varieties failed to produce any nodules in this soil even when heavily inoculated with cultures which produced abundant nodules in other soils tested in comparison. Heavy liming of the soil extract at the rate of about 6,000 pounds to the acre gave a fair growth of the bacteria, and corresponding to this result one pot that received lime in the same proportion produced a well-noduled plant. In this same soil without lime and without inoculation cowpeas formed nodules abundantly.

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[a] See Rhode Island Agr. Exp. Sta. Rept., 1896, p. 256; also Bul. 96 of same station, 1903.

[b] Deutsche Landw. Presse, vol. 32, 1905, p. 799.

A soil from Lanham, Md., gave an extract which as a culture medium was distinctly unfavorable to the growth of red clover bacteria, and plants of red clover in this soil made a very sickly growth, producing few nodules. A few pots were boiled by immersing in a tub of water and blowing live steam into the water; the soil was of a sandy character, so that there was little danger of puddling from this treatment. The growth of red clover was markedly improved in the boiled series and the roots were well noduled after inoculation. The extract of this soil, sterilized by boiling, had proved an unfavorable medium for the growth of the bacteria. It is probable, therefore, that boiling the soil changed the character of the soil solution at least in regard to the unfavorable materials or conditions which had inhibited bacterial activity.

Soil from Lanham, Md., was further tested with alfalfa, treating the soil in three ways: (1) Lime at the rate of one ton to the acre; (2) lime and one-fourth humus, composed of leaf mold; (3) humus. The combination of lime and humus gave the greatest growth and most abundant nodule formation. With humus alone the growth was especially inferior and nodules were lacking even where the soil was inoculated. Inoculation doubled the number of nodules where lime was used and increased them six times where both lime and humus were used.

Effect of humus and lime upon the growth of alfalfa in soil from Lanham, Md.

[Ten plants in each series.]

Treatment.	Average (green) weight of plant.	Average number of nodules.
Lime alone:	*Grams.*	
Uninoculated	0.86	0.5
Inoculated	1.56	1.0
Humus alone:		
Uninoculated	.5	0
Inoculated	.8	0
Humus and lime:		
Uninoculated	.73	1.4
Inoculated	2.45	8.6
No lime; no humus:		
Uninoculated	(*)	.3
Inoculated	(*)	.5

*Not weighed; growth so small as to be a practical failure—2 to 5 inches as compared with 12 to 18 inches high in the limed pots or those containing lime and humus.

The sterilized extracts of soil from this series of pot tests gave results seemingly contradictory when used as culture media for bacteria in comparison with the growth of plants and nodules. The extract of untreated soil gave a growth of the alfalfa bacteria which might be designated "fair," while with lime, with lime and humus, and with humus alone the extracts gave uniformly a growth which might be designated "excellent." In the pots, however, no nodules developed in the humus series. The sterilization by heat of the extract from a soil containing such a relatively large quantity of humus, one-fourth by volume, probably changes the material so that the solution becomes favorable to the growth of the bacteria, but this material in the soil pots, not heated, remains unchanged and may in this form be unfavorable to the activity of the bacteria introduced into the soil.

Aside from the effect of the soil on nodule formation there seems to exist a marked difference among different varieties of legumes in their susceptibility to infection—that is, their readiness to form nodules. With a similar soil and one which is favorable to the growth of the respective species, nodules will occur in abundance upon one species or variety and another species or variety will exhibit none or only a few. This is particularly noticed with varieties of soy beans and with certain species of Phaseolus, the Lima bean (*Phaseolus lunatus*) and some varieties of soy beans being very difficult to inoculate. It would seem that such species were actually resistant to infection, especially in soils rich in nitrogenous matter.

EFFECT OF HEAVY INOCULATION.

The decided advantage of very heavy applications of pure cultures reported from some field and pot experiments and reports of other experiments showing that cultures diluted to almost infinitesimal limits gave as good results as undiluted cultures seem at first diametrically opposed. Greenhouse tests seem to confirm the beneficial effect to be expected from heavy inoculation provided excessive quantities of culture are applied. The following experiment is fairly representative:

Effect of light and heavy inoculation of garden peas on soil from Blue Hill, Me.

Treatment.	Number of plants grown.	Number of plants with nodules.	Average number of nodules.
Check, without inoculation	11	2	1
Light inoculation	9	6	3
Heavy inoculation	6	6	7

It is here shown that heavy inoculation not only doubled the average number of nodules upon inoculated plants, but insured all plants becoming infected; while with light inoculation in this unfavorable soil one-third of the plants failed to produce nodules, and with no inoculation nodules were almost entirely lacking.

Garden peas in a garden soil naturally inoculated showed the following difference where a heavy inoculation was made:

Effect of heavy inoculation of garden peas on a naturally inoculated garden soil.

Treatment.	Number of plants grown.	Number of plants with nodules.	Average number of nodules.
Untreated	15	15	6¼
Heavy inoculation	18	18	19

It is highly probable that the cause of the formation of more numerous nodules in some cases is due not to the great numbers of bacteria which are introduced into the soil but to the quantity of culture medium introduced, which renders the soil solution a materially richer food for the active growth of the nodule organisms. Thus, if a few bacteria are brought into a favorable soil they will multiply rapidly, and it is of no particular moment in such a case whether a very few or a large number of bacteria are originally introduced. If, however, enough culture is added so that the soil solution is appreciably improved in food material for both plant and bacteria, then growth will be much more active and nodule formation will be greater. On this hypothesis the two results are not at all at variance, the effects produced depending on the soil conditions encountered by the bacteria and the crop.

The suggestion in a former bulletin[a] that the slight varietal characteristics exhibited by legume bacteria can be readily broken down by cultivation on synthetic nitrogen-poor media seems to hold only for bacteria isolated from plants physiologically related to the subsequent hosts.[b]

A typical experiment is given below:

Number of nodules produced on two varieties of mung bean (Phaseolus viridissimus and P. calcaratus) by cultures from various hosts.

[Ten plants in each of seven series.]

Variety of mung bean.	Bacteria from cowpea.	Bacteria from soy bean.	Bacteria from P. viridissimus.	Without bacteria.
Phaseolus viridissimus	*1	0	77	0
Phaseolus calcaratus	0	0	88	0

*Fifteen plants in this series.

It may be added that the soil used was unsterilized garden soil, naturally inoculated for our common bean, *Phaseolus vulgaris*, so that the uninoculated check may be said to serve as a cross-inoculation with these organisms also. This theory of varietal differences of nodule-

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[a] Bul. 71, Bureau of Plant Industry, 1905, pp. 25–27.

[b] See Schneider, Ill. Agr. Exp. Sta. Bul. 29, 1894, p. 301.

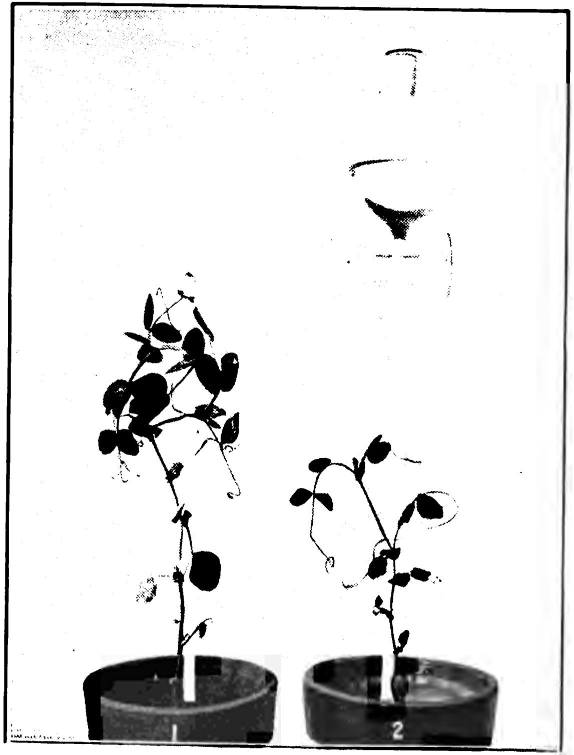

FIG. 1.—POTS OF GARDEN PEAS, SHOWING THE EFFECT OF AERATION ON GROWTH.

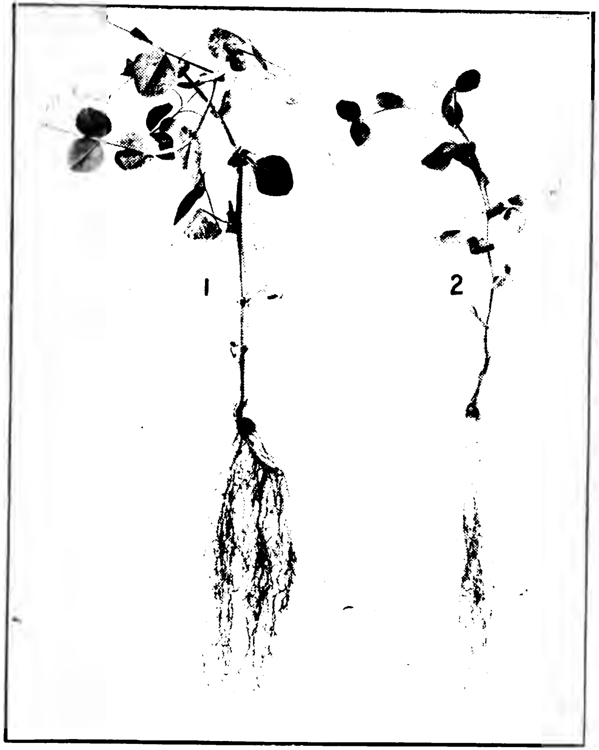

FIG. 2.—THE GARDEN PEAS ILLUSTRATED IN FIGURE 1, SHOWING THE EFFECT OF AERATION ON NODULE FORMATION.

forming bacteria is closely in accord with field results reported by various investigators, among whom perhaps Hopkins[a] is best known for his observations upon the cross-infection between sweet clover and alfalfa and the absence of such cross-infection between many other legumes.

EFFECT OF AERATION.

The aeration of soil for securing plentiful nodule formation is an important if not a determining factor. The Ontario Agricultural Experiment Station (Report for 1905, p. 39) reports a decided gain from aeration in the case of a leguminous crop (peas) and no gain from the aeration of a wheat crop. In the former case the result may be either a direct one, affecting the activity of the nodule bacteria, or it may be that because of the abnormal growth of the host plant the bacteria are unable to penetrate the root.[b]

Our own experiments upon the effect of aeration are in accord with those quoted in regard to the legumes, and the following is typical: A light sandy soil, moderately limed, was used and the legume selected was the garden pea. The aerated series was started in ordinary unglazed 4-inch pots, seedlings dipped in liquid culture when placed in position, pots watered with a fine sprayer, using tap water. The surface of the soil was frequently stirred. The nonaerated series was made up of an equal number (6) of glazed 4-inch pots with bottoms plugged with paraffined cotton and the tops covered with paraffined paper to reduce aeration. A separatory funnel (shown in Pl. IX, fig. 1) was thrust into each of these pots to admit water for growing the peas, and after inoculating as with the other series the seedlings were introduced through a hole in the paraffined paper. After a month's time the roots of the plants were examined, with the following results:

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[a] Ill. Agr. Exp. Sta. Bul. 94, 1904, Nitrogen Bacteria and Legumes.

[b] Livingston (Bot. Gaz., XLI, 2, 1906, p. 143; see also Bureau of Soils Bul. No. 28) reports a close relation between the growth of roots and tops in wheat, due to a difference of soil, which he explains as follows: "It would seem that the poor soil by inhibiting branch growth and causing the enlargement of cortical cells may render the root system unable to carry on an adequate amount of absorption for normal growth." If this effect upon the cortical cells should be found to hold true with the legumes, it might have a direct bearing upon the ability of the bacteria to penetrate and form nodules. The enlargement of the cortical cell is regarded as a phenomenon of age, and it is reported (Maria Dawson, Phil. Trans. Roy. Soc. London, Ser. B, vol. 192 (1899), p. 24) that infection takes place most readily in quite young radicals.

Aside from the effect of root development upon nodule formation, there is a possible interaction resulting in some way in greater root development where inoculation takes place. In many cases which have come to notice where inoculated plants had shown a superior development of the root system, it seemed at least justifiable to regard the inoculation as assisting in providing the conditions favoring a healthy root growth. In Bulletin No. 237 of the Cornell Agricultural Experiment Station, page 165, it is noted that where no nodules occur the roots of alfalfa are simpler and not so ramifying as where nodules occur.

Treatment.	Nodules on plant No.—						Total.
	1.	2.	3.	4.	5.	6.	
Aerated	4	20	25	8	26	4	8
Nonaerated	0	1	2	0	0	0	

See Plate VIII, figure 2, noting the difference in the plants in favor of the noduled (aerated) plant.

ASSOCIATIVE ACTION OF BACTERIA.

Undoubtedly the pure cultures of nodule-forming bacteria are subject to contamination when planters, using convenient rather than bacteriological methods, prepare quantities sufficient to inoculate their seed. Yet the purity of a culture immediately before it is applied to seed or introduced into the soil of a cultivated field is unimportant, provided there is present a sufficient number of virile nodule-forming bacteria.

Preliminary experiments at Washington some years ago indicated that in the nitrogen-poor solution generally used the nodule-forming bacteria would develop much more rapidly than other bacteria or contaminating yeasts and molds. It might be expected that different localities would have different contaminating forms, and during the past year samples of cultures have been obtained from farmers in various parts of the United States immediately before the seed or soil was treated. Investigation of contaminating forms isolated from these cultures shows that there are certain forms which inhibit the growth of the nodule organism when both are grown for some time in the usual nitrogen-poor solution.[a] Using an extract of a favorable soil as a culture medium, results very nearly parallel for the competing bacteria were secured, though one organism which resembles *Bacillus coli* checked the growth of *Pseudomonas radicicola* in the synthetic medium and not in the soil extract. The relative virility of the competing cultures at the time they begin their struggle is an exceedingly important factor in determining which species of bacteria survives.

Extended experiments on the interaction of groups of bacteria in various media must be carried on before it will be possible to determine all of the conditions incident to success or failure in legume inoculation.[a] It is safe to assume, however, that the action of a bacterium in the soil is conditioned not only by the chemical and physical characteristics of the soil solution at a particular time, but also, and perhaps essentially, by the biologic conditions obtaining in that soil.

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[a] In view of this fact, the reason for our present method of distribution is apparent. A relatively large amount of pure liquid culture used as a "starter" cuts down the chances of ruining the farmer's culture through the competition of contaminating forms; the time required to fill the solution with the organisms is shortened, and, as shown subsequently, the quicker the bacteria can be introduced into the soil and make use of the soil solution as a culture medium the less becomes the danger from competition.

SUMMARY.

1. Lime is of decided benefit in obtaining successful inoculations of legumes in some soils. These soils often show an acid reaction to litmus.
2. Soil extracts serving as culture media often indicate the probable success of inoculating a leguminous crop. This, however, may not always hold true.
3. At least during the first season's growth no general cross-inoculation takes place. Bacteria from one host may, however, inoculate a physiologically related species.
4. Heavy inoculation by a pure culture increases nodule formation if the soil solution is enriched by the excess of culture medium; however, in a favorable soil a light inoculation well distributed is as effective.
5. Thorough aeration is favorable to nodule formation.
6. Whether in a synthetic medium or a natural soil solution, the functions of a bacterium are influenced by the associative or competitive action of the various groups of organisms with which it comes in contact, as well as by the nature of the culture material.

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[a] See Maria Dawson, Phil. Trans. Roy. Soc. London, Ser. B, vol. 193 (1900), p. 65.

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[Continued from page 2 of cover.]

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